A COMPARATIVE STUDY ON SEED HETEROMORPHISM IN *ACHNATHERUM BRACHYCHAETUM* (GODR.) BARKWORTH AND *NASSELLA CLARAZII* (BALL) BARKWORTH

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ABSTRACT

Seed heteromorphism related to chasmogamous (CH) and cleistogamous (CL) seeds can have different ecological significance in species with different functional characteristics, competitive ability and palatability. Punagrass [*Achnatherum brachychaetum* (Godr.) Barkworth], an invasive, perennial grass is common in grasslands of Argentina and it is an aggressive weed in other parts of the world. Flechilla grande [*Nassella clarazii* (Ball) Barkworth] is a palatable perennial grass associated with the dominant "climax" vegetation in grasslands of Argentina. Seeds of the two grasses were collected from grasslands of Argentina, and growth chamber and greenhouse experiments were conducted to determine: 1) germination, dormancy breaking, and mass of CH and CL seeds of the two species 2) effects of contrasting range condition on germination and seed mass of punagrass, 3) the relative fitness of plants from CH and CL seeds, 4) if contrasting range condition affect fitness of CH plants of punagrass, and 5) the effect of maternal nutrient environments on CH and CL seeds and on fitness in the two species. Small CH seeds of high dispersal potential were less dormant than large CL seeds of low dispersal potential in punagrass. CH and CL seeds of flechilla grande had similar mass, germination, and response to dehulling. CL seed size and CL seed production of punagrass increased with good range condition. Increasing the maternal, nutrient environment enhanced germination of CH seeds, CL seed size, growth rate, development, biomass and seed production more in punagrass than flechilla grande. Under low nutrient conditions, flechilla grande produced a few large CH seeds. CH progeny of punagrass grew fast and developed rapidly as compared to CL progeny, which in turn produced many CH seeds. CH and CL seeds of flechilla grande had similar contribution to the fitness of adult plants. In both species, the ecological significance of having seed heteromorphism is that sibling competition is probably reduced by having more diverse offspring. Heavy grazing of competitive species such as flechilla grande may favours species as punagrass with many small CH seeds, high potential for colonization as well as large CL seeds for persistence in the seed bank and seedling competition.

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1. INTRODUCTION

Punagrass [*Achnatherum brachychaetum* (Godr.) Barkworth] and flechilla grande [*Nassella clarazii* (Ball) Barkworth] are cool-season, perennial grasses from grasslands of South America. Both species have seed heteromorphism associated with aerial, chasmogamous (CH) seeds from open and potentially outcrossed flowers on terminal panicles, and cleistogamous (CL) seeds from closed, self-fertilized flowers on axillary panicles enclosed within leaf sheaths (Cano and Eilberg, 1969; Eilberg, 1974; Cano, 1988). Punagrass is unpalatable and grows primarily in small patches beneath trees on loamy soils (Llorens, 1995). Punagrass, an invasive species, is common in fertile, disturbed areas, and in overgrazed paddocks (Ares *et al*, 1970b; Cano, 1988; Llorens, 1995; Gardener and Sindel, 1998). Flechilla grande is a palatable grass associated with the dominant "climax" vegetation, and grows on open areas; its abundance is reduced by heavy grazing (Llorens, 1995; Busso, 1997). It is more competitive than unpalatable *Stipa* species when protected from grazing, reducing their biomass and seed production (Moretto and Distel, 1997).

Plant responses to varying environmental conditions often involve mechanisms that increase fitness by optimising reproductive output under different environments. In general, under relatively poor environmental conditions for growth, the greatest fitness results from producing a few large CL seeds; however, if conditions are favourable for growth, CH seed production is enhanced (Schoen and Lloyd, 1984; Quinn, 1998). Seed heteromorphism patterns and position-dependent variation in seed mass, number, germinability and viability can be altered by environmental conditions of the maternal plant (Cheplick, 1996b; Cheplick and Sung, 1998). Furthermore, abiotic and biotic stresses can shift the mating system toward more CL flowers and seeds (Bell and Quinn, 1987; Le Corff, 1993; Bennington and McGraw, 1995; Culley, 2002; Steets and Ashman, 2004). In many species with seed heteromorphism, differences in germinability

also exist between dispersed and non-dispersed seeds (Venable and Lawlor, 1980; Cheplick, 1996a; Cheplick and Sung, 1998). Seeds with low dispersal potential are generally more dormant than seeds with high dispersal potential (Venable and Lawlor, 1980; Cheplick, 1996a; Olivieri, 2002). Moreover, in species with seed heteromorphism, larger seeds are correlated with better seedling survival and competitive ability (McNamara and Quinn, 1977; Cheplick and Quinn, 1982; Waller, 1984; Cheplick, 1994; Cheplick and Wickstrom, 1999).

The focus of this research was to study seed heteromorphism, specifically CH and CL seeds in punagrass and flechilla grande. The null hypothesis of this research was that seed heteromorphism plays similar roles in the fitness of the two species with different functional and ecological characteristics. The following questions were addressed:

1) Is seed germinability different between CH and CL seeds of punagrass and flechilla grande?

2) Are germinability, mass and viability of punagrass seeds affected by the condition of the range in which they are produced?

3) What are the relative contributions of plants derived from CH and CL seeds to the fitness of punagrass and flechilla grande?

4) Do nutrient environments of maternal plants lead to different fitness between CH and CL seeds and between punagrass and flechilla grande?

Information from these studies will improve the understanding of seed heteromorphism and its evolutionary and ecological significance. Results from comparable studies on the fitness of punagrass and flechilla grande under contrasting maternal conditions will allow formulating recommendations for the management of grasslands in Argentina aiming at the control of unpalatable species such as punagrass and restore climax dominant, palatable species such as flechilla grande.

2. LITERATURE REVIEW

2.1 Semi-arid Grasslands in Central Argentina

2.1.1 Distribution

The Caldenal or southern espinal is a xerophyllous woodland that extends over an elongated area in central Argentina, which interposes itself in a wide band between the prairies of the pampas and the Monte semi-desert (Cabrera, 1971; Mares *et al*, 1985). The woodland vegetation consists of an herbaceous layer with a discontinuous cover of caldén (*Prosopis caldenia* Burkart), a mycrophyllous, deciduous, leguminous tree. The Caldenal covers approximately 40,000 km^2 (Cabrera, 1971). The main economic activity in the Caldenal is cattle production on natural vegetation, and in some areas the natural vegetation has been replaced by pastures of weeping lovegrass [*Eragrostis curvula* (Schrad.) Ness], alfalfa (*Medicago sativa*) or annual crops (Cano, 1988). From the end of Pleistocene to the beginning of colonization by European settlers, large herbivores were scarce in Argentina (Bucher, 1987). Since the introduction of the domestic livestock during the 20^{th} century, rangelands have been overgrazed, and interactions with wood harvesting, alteration of fire regimes and above-average rainfall have promoted profound changes in the vegetation (Distel and Bóo, 1995; Llorens, 1995).

2.1.2 Physical environment

The climate is semiarid characterized by hot summers and cold winters; precipitation amounts are highly variable among seasons. The mean annual temperature is 16°C, the annual precipitation averages 608 mm, and the annual potential evapotranspiration (Thornthwaite method) is 815 mm, yielding an annual water deficit of 207 mm (INTA *et al*, 1980; Roberto *et al*, 1994). The mean temperature of July (the coldest month) averages 8 °C while temperatures average 24 °C in January (the warmest

month) (INTA *et al*, 1980). Absolute maximum and minimum temperatures of 42.5 °C and –12.8°C, respectively, have been recorded. The frost-free period averages 200 days. Variability in the precipitation is very large temporally (total monthly and total annually) and spatially, with most precipitation received in spring and fall (INTA *et al*, 1980; Gatto-Caceres and Dornes, 1996). Although droughts can occur during winter, those of greatest intensity are in late spring and summer (November through February) (INTA *et al*, 1980).

Soils in the grassland area of this study are Entic haplustolls or Molisolls of sandy to silty clay loam texture. These soils are well drained with low or moderate organic matter content and with a petrocalcic horizon from 0.5 to 1.5 m (INTA *et al*, 1980). The parent material of the soils is composed of late pleistocenic and holocenic eolian sediments (loess) (INTA *et al*, 1980). Generally, forests and associated grasslands are in lowlands (deeper and loamy-sand soils), shrublands are on slopes (deeper than uplands and sandy soils) and grasslands with tree encroachment are on uplands (shallower and sandy-loam soils). Soil nutrients are greater in burned pastures or under the canopy of caldén trees than outside the influence of the trees or unburned pastures (Castelli and Lazzari, 2002). Total and available nitrogen, total and available phosphorus, total and available sulphur, and pH increased in soils after burning (Castelli and Lazzari, 2002). Soil texture (silt + clay) and tree coverage are positively correlated with soil organic matter (Buschiazzo *et al*, 2004). Analysis of soil temperatures showed that minimum temperatures were higher, and the temperature amplitude was lower under the canopy than outside the canopy (Buschiazzo *et al*, 2004). Air temperatures and the soil water content are less variable under the tree canopy than in open areas (Llorens and Frank, 1999).

2.1.3 Vegetation

When domestic livestock were introduced, the vegetation of the Caldenal was savanna with caldén trees dispersed in a matrix of short grasses (Llorens, 1995; Dussart *et al*, 1998). The forest was located mostly in valleys, and grasslands were on dunes and uplands (INTA *et al*, 1980). The invasion of pristine grasslands by woody plants shifted the savanna into stable woodlands or shrublands (Distel and Bóo, 1995; Llorens, 1995; Dussart *et al*, 1998). The herbaceous layer was dominated by short-grasses, and mid-

grasses grew in small patches beneath trees (Llorens, 1995). The short grasses in this region are generally of good forage value and are presently they are almost common in open areas of properly managed grasslands (Llorens 1995). The mid-grasses have little or no forage value and are most common in the shade of woody vegetation and in overgrazed areas. A replacement of species in the herbaceous layer has also occurred, as significant portions of the grasslands have been invaded by unpalatable mid-grasses, which has been attributed to by the interaction of overgrazing, fire control and above-average rainfall during the last century (Distel and Bóo, 1995; Llorens, 1995; Busso, 1997; Llorens and Frank, 1999).

According to Distel and Bóo (1995) and Llorens (1995), five main plant communities can be identified in the herbaceous layer of the Caldenal: *Poa*, Flechilla, Mixed grass, Mid-grass and Annuals. The *Poa* and flechilla communities are considered excellent to good range condition, whereas that Mid-grass and Annuals are classified as fair to poor condition. The Mixed grass community can be categorized between good and fair range condition. The Mixed grass and the Mid-grass communities are more frequent today (Llorens 1995).

The *Poa* community is a "climax" community, dominated by the short, cool season grass unquillo (*Poa ligularis* Ness ex Streudel). Associated species vary with soil texture. Flechilla grande, tembladerilla (*Briza subaristata* Lam.), flechilla negra (*Piptochaetium napostaense* Speg.), and flechilla fina (*Stipa tenuis* Philippi) grow on loamy soils while pasto hilo (*Poa lanuginosa* Poiret), penacho blanco [*Bothriochloa springfieldii* (Bould.) Parodi], and the perennial forb te indio [*Thelesperma magapotamicum* (Spreng.) O. Ktze.] grow on sandy soils (Llorens, 1995).

The Flechilla community is dominated by flechilla negra, with accompanying species such as flechilla fina and California cottontop [*Digitaria californica* (Benth.) Henrard] in loamy soils and pasto hilo in sandy soils. All species provide good forage.

Mixed grass community are co-dominated by flechilla negra and warm season grasses, most often sand dropseed [*Sporobolus cryptandrus* (Torr.) A. Gray] and California cottontop. A few isolated mid-grasses occasionally occur under the trees.

The Mid-grass communities is dominated by either finestem needlegrass (*Stipa tenuissima* Trinius), paja blanca (*Stipa gynerioides* Philippi) or both. These species have

poor forage quality and they are normally accompanied by other undesirable mid-grass species such as punagrass, paja dura (*Melica bonariensis* Parodi) and balsamscale [*Elyonurus muticus* (Spreng.) O. Kuntze].

The annual communities are dominated by aggressive annual species, such as sanbur (*Cenchrus pauciflorus* Bentham), Russian thistle (*Salsola kali* L.), and cebadilla (*Bromus brevis* Ness).

2.2 Plant Description and Ecological Characteristic of the Species Studied

2.2.1 Punagrass (*Achnatherum brachychaetum* (Godr.) Barkworth)

Punagrass is native to Argentina and Uruguay (Parodi, 1964; Rosengurtt, *et al*, 1970). It is an invasive species in pastures and this grass is abundant in overgrazed grasslands of Argentina (Caro and Sanchez, 1971, Cano, 1988). In Argentina and the United States, punagrass has been reported as a serious weed in alfalfa pastures (Fuller, 1961; Ares *et al*, 1970b; Rodriguez, 1983; Canevari and Viss, 1998). In the United States, punagrass is listed as a noxious weed, occurring in Arizona, California and Oregon (USDA 1953; Barkworth 1993; USDA 2005). In Australia, punagrass is naturalized; it is considered an agricultural weed and an exotic species that invades native vegetation (Gardener and Sindel, 1998). The potential distribution of punagrass in Australia has been estimated at 0.6 million ha with a large area of New South Wales at risk (McLaren *et al*, 1998). This species also grows in Tasmania (Morris, 1983).

Punagrass is a dominant or co-dominant in overgrazed lowland areas of the Caldenal (Cano, 1988). The grass is common in the shade of woody vegetation and in overgrazed areas (Llorens, 1995). In Australia, several species of *Achnatherum* and *Nassella*, including punagrass, generally invade degraded vegetation with a history of human disturbance, and lands with highly fertile soils often previously used for grazing or farming (Gardener and Sindel, 1998).

Punagrass is a cool-season, mid-height perennial bunch grass (Caro and Sanchez, 1971; Cano, 1988). In the Caldenal, punagrass grows primarily in small patches beneath the trees and on loamy soils (Llorens, 1995). This is an unpalatable species of little forage value, with high lignin and cellulose content (Cano, 1988). Unpalatable mid-grasses such as punagrass have inferior competitive ability compared to palatable

species (Moretto and Distel, 1997). Unpalatable species can acquire a competitive advantage after disturbances in terms of biomass production, tiller recruitment and seed production when neighbouring palatable species are defoliated (Moretto and Distel, 1999). Moreover, unpalatable mid-grasses are more dependent on greater precipitation. Increases in seedling mortality, reductions in plant basal area and declines in shoot production of these species were associated with years of below average precipitation (Moretto and Distel, 1998; 1999). High positive correlations between rainfall and cover of these species were found over 11 years (Llorens and Frank, 1999). Punagrass is adapted to lowlands of high fertility, with lower leaf nitrogen and phosphorus use efficiency than species adapted to low fertility conditions on uplands (Distel *et al*, 2003).

Punagrass has heteromorphic seeds associated with cleistogamy, with chasmogamous (CH) flowers from apical panicles and cleistogamous (CL) flowers from axillary panicles within leaf sheaths on each stem node (Eilberg, 1974). In Argentina, Punagras flowers in late November, and sets seeds in December (Cano, 1988). In California, CH flowers of punagrass emerge in April with seed maturation in July, whereas CL flowers begin blooming in June and continue through November (Canevari and Viss, 1998).

Up to 15, one-flowered spikelets were found in the axillary prophylles of the basal nodes in Punagrass (Cano and Eilberg, 1969). The CH seeds of the apical panicles are uniform in size, whereas that CL seeds are heterogeneous in size and shape (Eilberg, 1974). CL seeds develop in all nodes of the stem (Eilberg, 1974; Cano, 1988).

Optimum germination conditions for punagrass are 20/15 °C, 9/15 hours in darkness (Eilberg and Soriano, 1972). Germination of 40 % for CH seeds in the field was reported for punagrass when seeds were placed on the soil surface, after harvest in the fall (Eilberg and Soriano, 1972). When CH seeds were buried at 15 cm in the soil to simulate tillage before sowing alfalfa, germination was 22% (Eilberg and Soriano, 1972). Thus, a portion of CH seeds can germinate soon after seed set in the field and dormancy can be broken by field conditions.

CL seeds germinated to10 or 5-8 % in the field on soil surface or when buried in the soil, respectively, after harvest in the fall (Eilberg and Soriano, 1972). After two years, CL seeds that remained on soil surface or were buried at the 15 cm depth had 60%

to 80% dormancy, respectively (Eilberg and Soriano, 1972). Dormancy of CL seeds was partially broken, and more seeds germinated in the laboratory when CL seeds remained in the soil at least one year (Eilberg and Soriano, 1972). Most seedlings of punagrass in alfalfa originated from CL seeds (Ares *et al*, 1970b).

On the other hand, the greatest seedling emergence and establishment of unpalatable *Stipa spp*. in microsites without competition from palatable species have been related to the higher soil temperature and water content in those microsites (Moretto and Distel, 1998). In undulated soils, a higher density of Punagrass seedlings emerged from seeds located in small depressions, with greater availability of water (Ares *et al*, 1970a).

2.2.2 Flechilla grande (*Nassella clarazii* (Ball) Barkworth)

Flechilla grande is native to Argentina, Uruguay and Chile (Correa, 1978; Cano, 1988). This species is associated with the dominant climax vegetation of grasslands in western and southern areas of Buenos Aires (Cabrera, 1970) and in the Caldenal (Cano, 1988; Llorens, 1995; Busso, 1997). The abundance of flechilla grande is low after heavy grazing (Busso, 1997).

Flechilla grande is a cool-season, perennial bunch grass (Cano, 1988). Plants regrow in the fall, flower in spring, and fruit in late spring-early summer (Cano, 1988). In central Argentina, flechilla grande grows in the Caldenal, in upland grasslands and *Larrea* shrublands (Cano, 1988). Plants occupy open areas with loamy soils (Llorens, 1995).

Flechilla grande is a palatable, highly preferred species with good forage quality (Cano, 1988). Flechilla grande has a greater competitive ability in the absence of defoliation by reducing biomass and seed production of nearby unpalatable *Stipa* species (Moretto and Distel, 1997). Moreover, flechilla grande tolerates shortage in water availability and defoliation. Flemmer *et al* (2002) showed that growth of flechilla grande was stimulated by defoliation even under water stress. One defoliation to a 5-7 cm, stubble height at the vegetative stage under water stress and irrigated conditions, and two defoliations (vegetative + internode elongation stages) under natural rainfed conditions, increased tiller number (Flemmer *et al*, 2002). Moreover, flechilla grande had similar or higher nitrogen uptake than *S. tenuis* and *S. ambigua* (Saint-Pierre *et al*,

2004). Higher nitrogen uptake in flechilla grande thus appear to be one of the mechanisms contributing to its greater competitive ability compared to other species (Saint-Pierre *et al*, 2004).

Flechilla grande exhibits seed dimorphism associated with chasmogamy and cleistogamy (Cano 1988). Plants have large aerial chasmogamous (CH) seeds from apical panicles and two or three large cleistogamous (CL) seeds from axillary panicles within leaf sheaths at the base of the plant. CH seeds have a hygroscopic awn that is 6-8 cm in length (Cano 1988). Flechilla grande produces more CH seeds in ungrazed than in grazed conditions (Distel and Klich, 1995).

Optimum conditions for seed germination of flechilla grande are 25/15 °C, 9/15 hours light/darkness (Cabeza *et al*, 1999). Removal of palea and lemma increased the germination percentages in CH seeds of flechilla grande after six months of dry after-ripening. The germination percentage after hull removal was similar to viability percentages, indicating that dormancy in CH seeds is controlled by the hulls (Cabeza *et al*, 1999). Hulled CH seeds of flechilla grande had less germination after two years of storage compared to fresh seeds (Cabeza *et al*, 1999). Application of potassium nitrate and giberellic acid increased the germination of the hulled seeds, which was similar to that of the dehulled seeds (Cabeza *et al*, 1999).

2.3 Seed Heteromorphism

2.3.1 Seed number and seed mass

Seed heteromorphism is the variation in size, shape, dispersability and dormancy of seeds or dispersal units (Silvertown, 1984). Genetic variation may take the form of a species-specific heteromorphism, whereby distinct seed types, often differing in seed number and seed mass, mature at different positions on the maternal plant (Silvertown, 1984; Venable, 1985; Cheplick and Clay, 1989; Kigel, 1995; Cheplick, 1996b; Gutterman, 2000).

Plants are expected to alter seed number before altering seed mass if resource levels vary because more seeds represent more offspring (Smith and Fretwell, 1974). On the other hand, larger, better-provisioned seeds have a greater chance of establishing (Leishman *et al*, 2000). The key prediction of the Smith-Fretwell model (1974)

regarding to producing more seeds of the same size is limited by the observed variation in seed mass within a species, which can be attributed to the limited capacity of the machinery of seed provisioning, the variability of competition the seedlings are exposed to or frequency –dependent effects (Leishman *et al*, 2000). Resource constraining that occur during seed provisioning limit the ability of plants to control the size of individual seeds and can cause trade-offs between seed number and mass (Vaughton and Ramsey, 1998).

Seed polymorphism is important in the recruitment of new individuals into the population. Within-species studies have found that seedling size often increases with seed mass, affecting fitness via the subsequent growth and survival of individuals (Stanton, 1984; Wulff, 1986; Vaughton and Ramsey, 1998). Increased competitiveness may be the primary selection pressure responsible for large seed mass as predicted by models (Leishman *et al*, 2000).

If germination and establishment must take place in sites where intra-specific or interspecific competition is severe, there can be selection to increase the energy reserves within the seed, often resulting in large seeds with poor dispersal (Willson and Traveset, 2000) but with high probability for successful establishment close to the maternal plant (Waller, 1984; Cheplick, 1998; Cheplick and Wickstrom, 1999). Moreover, larger-seeded species are better equipped to emerge from deeper in the soil and litter (Cheplick and Quinn, 1987; Molofsky and Augspurger, 1992; Cheplick and Grandstaff, 1997; Kitajima and Fenner, 2000). On the other hand, seed dispersal in space is more effective when seed weight is low, as dispersed seeds are packed with lighter energy sources (Westoby *et al*, 1996; Rees, 1997).

2.3.2 Germination patterns

Pre- and post-maturation changes in seeds affect dormancy (Bewley and Black, 1994; Baskin and Baskin, 1998) and reflect variability in seed germination caused by morphological or physiological polymorphism (Fenner, 1985). One of the factors affecting polymorphism in germination is seed position on the maternal plant (Silvertown, 1984; Cheplick, 1996a; Gutterman, 2000). Furthermore, germination patterns can be influenced by environmental factors impacting the maternal plant (Weiss, 1980; Cheplick and Sung, 1998; Gutterman, 2000).

In many species with seed heteromorphism, there are also differences in the germination percentage and germination rate between dispersed and non-dispersed seeds (Venable and Lawlor, 1980; Cheplick, 1996a; Cheplick and Sung, 1998). Dispersed seeds are generally less dormant than non-dispersed seeds (Venable and Lawlor, 1980; Cheplick, 1996a; Olivieri, 2002).

A model developed by Nilsson *et al* (1994) regarding the evolution of seed dormancy /germination patterns for predictable environments shows how seed dormancy could improve maternal plant fitness when interactions between siblings have negative density-dependent impacts on each other's survival, growth, and fecundity. Another model describing the effect of seed dispersal and inbreeding on the evolution of seed dormancy, for a population with patchy spatial structure in a constant environment, predicts that, 1) dormancy increases as the dispersal potential of seeds decreases, and 2) inbreeding delays germination to avoid sibling competition for limited resources (Kobayashi and Yamamura, 2000). On the other hand, the Olivieri model (2002) of seed heteromorphism in a metapopulation indicates that dispersal and dormancy evolve as a response to variable environments. When fecundity is low enough that sites are not saturated immediately after recolonization, the evolutionary stable dormancy rate and dispersal rate can both decrease with increasing local extinction rates (Olivieri, 2002). This is consistent with the expectation that the main function of dispersal and dormancy are escape from local crowding (Olivieri, 2002). In agreement with theoretical predictions, the CL seeds of *Sporobolus vaginiflorus* and *Triplasis purpurea* that were least likely to disperse in space and most likely to experience sibling competition had low germination and reduced germination rate (Cheplick, 1996a).

2.4 Chasmogamous and Cleistogamous Mating System

2.4.1 Seed heteromorphism associated with cleistogamous and chasmogamous flowers

Most flowers of grasses are open for cross-pollination, and they are called chasmogamous (CH) flowers. However, in some grasses the flowers do not open and they are self-fertilized in enclosed flowers, which are called cleistogamous (CL) flowers (Campbell *et al*, 1983). Thus, plants can have mixed mating systems for bearing CH and

CL flowers on the same individual. A few grasses such as *S. subinclusus* and *Tetrapogon spanthaceus* produce only CL flowers (Uphof, 1938). CL flowers of grasses can be produced 1) within the leaf sheaths on upper portions of the plant, or near the ground (cleistogenes), 2) on subterranean stems (amphycarpy), and 3) enclosed by spikelet parts (Campbell *et al*, 1983). The Fig. 2.1 illustrates the different types of cleistogamy, as described by Campbell *et al* (1983). Most cleistogamous grasses retain the ability to produce chasmogamous spikelets on terminal emergent panicles. Seeds produced by CH and CL flowers may vary in size (Clay, 1983b) and dormancy (McNamara and Quinn, 1977; Bell and Quinn, 1985).

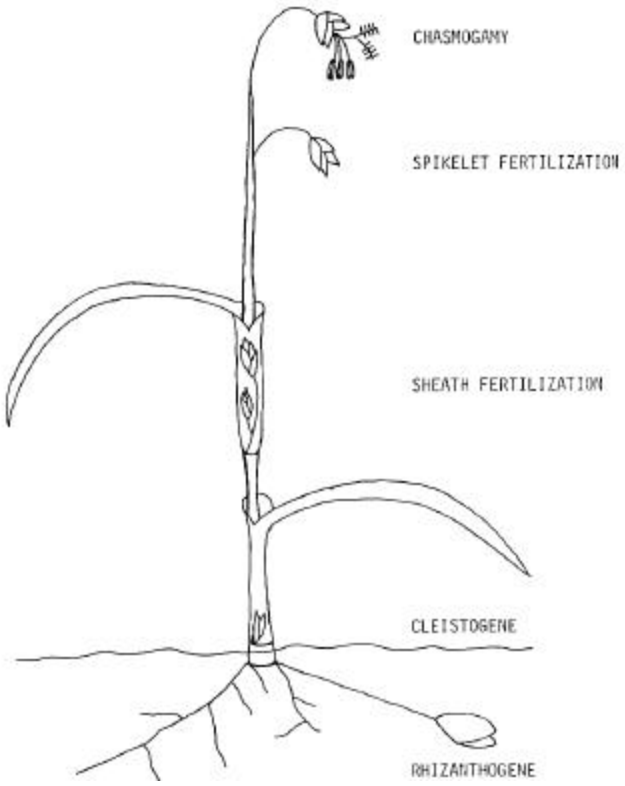

Figure 2.1 Schematic drawing of a grass plant showing chasmogamy and different types of cleistogamy. Drawing by C. S. Campbell in Quinn (1998).

Seed heteromorphism can be associated with seeds maturing in cleistogamous spikelets in a position-specific manner on axillary inflorescences enclosed within leaf sheaths (Cheplick and Clay, 1989; Cheplick, 1996b). Mass of seeds decreases from the lower to the upper nodes along a tiller (Cheplick, 1996b). This decrease in seed mass is

accompanied by an increase in seed number, and thus seed heteromorphism involves position-dependent variation in seed mass and seed number. The maximum number of seeds can be achieved in the lighter CH seeds of the terminal panicles (Cheplick and Sung, 1998). Seed heteromorphism patterns associated with cleistogamy have been observed in many perennial grasses (Cheplick and Clay, 1989) and an annual grass (Cheplick, 1996b). Dispersal ability decreases from top to bottom in CL seeds (Cheplick, 1998). Cleistogene production is usually associated with seed dimorphism and with specialised dispersal mechanisms (Campbell *et al*, 1983).

2.4.2 Differences in fitness between plants derived from cleistogamous and chasmogamous flowers

In species that possess seed heteromorphism associated with chasmogamous and cleistogamous flowers, floral type may have a large impact on the fitness of progeny (McNamara and Quinn, 1977; Cheplick and Quinn, 1982; Clay, 1983b). Moreover, Venable (1985) showed that seed specialization would occur more easily if there were trade-offs between seed types in different environments.

Although it is expected that progeny from outcrossed CH flowers have greater vigour than that from CL flowers because of the avoidance of inbreeding depression, this is not always true. The relative performance of outcrossed and selfed progeny must be evaluated under different environmental conditions (Quinn, 1998). Moreover, a clear fitness advantage for the CL progeny occurs when CL seeds are much larger than CH seeds (McNamara and Quinn, 1977; Weis, 1980; Cheplick and Quinn, 1982; Cheplick, 1994; Cheplick and Wickstrom, 1999).

Mixed mating systems may be selected for as a way to minimize sibling competition. More diverse offspring is predicted to confer greater ability for partitioning limiting resources (Cheplick, 2004). Rather, sibling competition because of the restricted dispersal of CL seeds reduced growth more than competition between unrelated plants, in accordance with the resource-partitioning hypothesis (Cheplick, 2004). Genetically variable offspring experienced less competition than genetically similar or identical offspring in *T. purpurea* (Cheplick, 2004).

Different success of CH and CL progeny has been observed in *Amphicarpum purshii* and *Microlaena polynoda* in which CL seeds are larger and heavier than CH

ones (Campbell *et al*, 1983). For *A. purshii*, the growth of plants from CH seeds was severely depressed in mixtures of plants from the two types of seeds at two densities in the greenhouse (Cheplick and Quinn, 1983). In the field, survival was greater for seedlings from CL seeds on dry and wet sites and at low and high densities. In addition, plants from CH seeds grew less and produced fewer seeds than from CL seeds (Cheplick and Quinn, 1982). More resource allocation to reproduction was noted for the plants of the selfed progeny in *A. purshii* (Cheplick and Quinn, 1982; Cheplick and Quinn, 1986). In *M. polynoda* most seedlings arise from the heavier CL seeds (Campbell *et al*, 1983).

In the perennial grass *Dichanthelium clandestine*, seeds produced by CH and CL flowers are equal in size, and differences in fitness arise mostly at the juvenile stage (Bell and Quinn, 1985). The percentage and rate of germination were higher in CL than in CH seeds, but seed viability did not differ. Seedling emergence was greater for CL progeny than CH progeny in the field and greenhouse (Bell and Quinn, 1985).

CL seeds were larger than CH ones in several species, and seedlings from CL seeds were larger and seedlings had higher survival than those from CH seeds (McNamara and Quinn, 1977; Cheplick and Quinn, 1982; Cheplick, 1994; Cheplick and Wickstrom, 1999). However, in *Impatiens capensis*, CH seeds are larger than CL ones; larger seedlings from CH seeds had greater survival and they were competitively superior to those from CL seeds under field conditions (Waller, 1984).

Plant fitness may be highly correlated with timing of reproduction (Rathcke and Lacey, 1985; Bennington and McGraw, 1995). Age at first reproduction is a life history trait that is thought to be subject to strong selection (Samson and Werk, 1986). Plants from CL seeds of *A. purshii* (McNamara and Quinn, 1977; Cheplick and Quinn, 1982) flowered sooner than those plants from CH seeds. However, plants from aerial CH seeds of *Commelina benghalensis* flowered sooner than those from subterranean CL seeds (Walker and Evenson, 1985).

2.5 Factors Affecting Chasmogamous and Cleistogamous Components of Reproduction Associated with Seed Heteromorphism

2.5.1 Proximate factors

2.5.1.1 Maturation position

Explanations for position based seed heteromorphism may involve differences in the timing and length of seed maturation. Resulting from the manner in which grass tillers grow by adding phytomers over time (Briske, 1991), the length of the seed maturation period varies with node position. Thus, a considerable amount of the variation in seed number and mass associated with node position may be a function of variation in the timing of reproductive development (Silvertown, 1984; Cheplick and Clay, 1989; Gutterman, 2000).

The position at which seeds mature on an individual can have also significant effects on their subsequent germination (Datta *et al*, 1972; Silvertown, 1984; Simpson, 1990, Gonzalez Rabanal *et al*, 1994; Kigel, 1995; Cheplick, 1996a; Gutterman, 2000). Germination increased with increasing node position in *T. purpurea* and CH seeds of the terminal panicles had the greatest germination (Cheplick and Sung, 1998).

2.5.1.2 Maternal nutrient environments

For several species with seed heteromorphism, environmental factors impacting the maternal plant affect the relative quantities of the different types and their relative mass (Weiss, 1980; Kawano *et al*, 1990; Cheplick, 1994; Kigel, 1995; Cheplick, 1996b; Cheplick and Sung, 1998; Gutterman, 2000), the balance between CH and CL seeds (Le Corff, 1993; Cheplick, 1994; Cheplick and Sung, 1998), and the magnitude of germinability of CH and CL seeds (Cheplick and Sung, 1998). The ability of maternal plants to alter the relative size and number of their offspring in response to the prevailing conditions could be critical to fitness (Venable, 1992; Kigel, 1995).

In general, under relatively poor environmental conditions for growth, the greatest fitness results from economically produced CL seeds; however, if environmental conditions are highly favourable for growth, the greatest fitness results from an increased production of the more costly CH seeds with high dispersal potential

(Schoen and Lloyd, 1984; Quinn, 1998). If resources are available, additional CH seeds are produced (Cheplick, 1989; Cheplick, 1994; Cheplick, 1996b; Le Corff, 1993; Cheplick and Sung, 1998). The greatest response in seed number to nutrients occurred in the uppermost nodes including CH seeds of the terminal panicles, but differed very little at the lower nodes in *T. purpurea* (Cheplick, 1996b; Cheplick and Sung, 1998).

Nevertheless, CL seed production increases with soil fertility in *Viola mirabilis* and *T. purpurea* (Mattila and Salonen, 1995; Cheplick and Sung, 1998). Nutrient availability did not alter CL seed production in *C. micans* (Le Corff, 1993). Mineral nutrients are clearly a major factor that can limit seed size and number (Parrish and Bazzaz, 1985). The production of a consistent number of large seeds at the basal nodes probably ensures some constant level of reproduction in an uncertain, stressful environment where nutrients may be in short supply (Schoen and Lloyd, 1984).

The addition of nitrogen fertilizers to maternal plants can decrease dormancy in many species (Fenner, 1991). Germination more than doubled when high levels of nitrogen and phosphorus were applied together to maternal plants of tomato (*Lycopersicum esculentum* Mill. Castellano*)* (George *et al*, 1980). CH and CL seeds from upper nodes had the greatest germination with increasing levels of nutrients for one *T. purpurea* population (Cheplick and Sung, 1998). Seeds matured in high-nutrient environments were heavier and had greater nutrient content (Parrish and Bazzaz, 1985; Vaughton and Ramsey, 1998) which together was suggested as the cause for the higher germination in *T. purpurea (*Cheplick and Sung, 1998).

About 60% of the grass species with cleistogamy are colonizers of disturbed or early successional habitats, and about 16 % of the rest are competitors (Campbell *et al*, 1983). Fast-growing species develop in unpredictable, but productive environments where disturbances occur, allowing rapid occupation of space (Grime, 1979). Conversely, plants of low-resource and stressful environments consistently grew slower than plants from more favourable environments (Lambers *et al*, 1998). In particular, grasses originating from nutrient-poor habitats tend to have a lower growth potential, when measured under optimal environmental conditions in the laboratory, than species from nutrient-rich habitats (Bradshaw *et al*, 1964). Slow growing species from nutrient-poor habitats are less sensitive to the variation in external supply than fast growing

species from nutrient-rich habitats (Garnier, 1998; Lambers *et al*, 1998). Late successional species reduced nitrogen to the lowest levels on infertile soils and had less vegetative growth than early successional species (Tilman and Wedin, 1991).

Success of plant invasion is influenced by: the number of propagules, the characteristics of the invading species, and the susceptibility of the environment to invasion (Crawley *et al*, 1996; Willamson and Fitter, 1996; Lonsdale, 1999). Moreover, invasibility is correlated with an increase in resource availability (Davis *et al*, 2000), dependent of the creation of bare ground and debilitation of preexisting vegetation (Thompson *et al*, 2001). Invasibility is also associated with regenerative traits such as seed size and germination characteristics (Burke and Grime, 1996). Species with small seeds, high resource demands, and potentially rapid growth are incapable of invading a closed cover of perennial vegetation (Hobbs and Atkins, 1988; Westoby *et al*, 1992; Burke and Grime, 1996). Large seeded species associated with perennial herbaceous communities on infertile soils have competitive advantages at later successional stages (Burke and Grime, 1996).

2.5.1.3 Plant size

The production of CH aerial and CL subterranean fruits of the amphicarpic annual *Gymnarrhena micrantha* Desf. (Compositae) was described as a dual strategy. CL seeds were produced according to a "pessimistic strategy" with seed production beginning concurrent with vegetative growth, while CH seeds followed an "optimistic strategy" as the vegetative stage precedes heavy fruiting (Zeide, 1978). The allocation of energy to larger, CL subterranean, propagules early and to smaller, CH aerial, propagules later if time and energy permit has been indicated for at least six different plant families including Gramineae (Cheplick and Quinn, 1982).

Indirect effects of environmental conditions on CH flower production have been found through their positive effect on plant size (Waller, 1980; Diaz and MacNair, 1998). In contrast, plant size had no effect on the type of reproduction in the tropical evergreen herb *Calathea micans* (Mathieu) Körn.; improvements in nutrient availability increased the production of new shoots that had a higher probability of producing CH inflorescences (Le Corff, 1993).

2.5.2 Ultimate factors: Ecological history of populations

Populations historically subject to high frequencies and intensities of disturbances can show great plasticity in their reproductive strategies, producing locally adapted progeny early and larger numbers of potentially more variable progeny later if time and resources are available (Quinn, 1998). In particular, the ratio of CH to CL seeds decreases with succession (McNamara and Quinn, 1977). The relative proportion of CH to CL flowers for species with mixed mating systems can vary greatly among populations of perennial grasses (Clay, 1983a; Bell and Quinn, 1987). For the first few years following a disturbance and removal of vegetation, *A. purshii* produced more CH than CL seeds; as succession progressed, plant cover and competitive interactions increased, and the vigour of plants declined. The ratio of CL to CH seeds increased to a point where more CL seeds than CH seeds were produced and finally to the point where no CH seeds and only a few CL seeds were produced (McNamara and Quinn, 1977). Thus, the relative levels of selfing and outcrossing change over a successional sequence.

Abiotic and biotic stress can shift the mating system toward greater production of CL flowers (Bell and Quinn, 1987; Le Corff, 1993; Cheplick, 1994; Bennington and McGraw, 1995; Culley, 2002; Steets and Ashman, 2004). In *Impatiens pallida* Nutt., outcrossed plants from stressful sites produced CL flowers earlier than outcrossed plants from non-stressful areas (Bennington and McGraw, 1995). An increased developmental rate allows plants to produce CL seeds before they succumb to stress (Bennington and Mc Graw, 1995). Populations of *Dichanthelium clandestinum* (L.) Gould that had higher allocation to CL reproduction at low soil moisture in controlled conditions, were also the ones exposed to the lowest soil moisture in the field (Bell and Quinn, 1987). In general, plants give priority to CL reproduction under stressful conditions because CL flowers are less expensive to make (Schemske, 1978; Waller, 1979). The differentiation between populations with respect to this trait may reflect an adaptive reduction in energy investment to reproduction with evolution favouring a more efficient process of seed production in more stressful sites (Bennington and McGraw, 1995).

A large amount of genetic variation was found in the degree of cleistogamy of *Danthonia spicata* (L.) Beauv., indicating the potential of this feature to respond to natural selection (Clay, 1982). In habitats where individuals are subject to marked

environmental fluctuations during their lifetime, or where progeny are subject to environments very different from those of their parents, it might be expected that selection is for adaptive plasticity in the breeding system (Quinn, 1998).

3. GERMINATION, DORMANCY BREAKING, AND MASS OF CHASMOGAMOUS AND CLEISTOGAMOUS SEEDS OF PUNAGRASS AND FLECHILLA GRANDE

3.1 Introduction

Pre- and post-maturation conditions affect seed dormancy change (Bewley and Black, 1994; Baskin and Baskin, 1998) and reflect variability in seed germination caused by morphological or physiological polymorphism (Fenner, 1985). Seed position on the maternal plant is one factor affecting polymorphism in germination (Silvertown, 1984; Cheplick, 1996a; Gutterman, 2000). Many species have two or more morphologically distinct types of diaspores produced by the same maternal plant with variation in size, shape, dispersability, and dormancy, and they are often associated with dimorphic flowers, e.g. cleistogamous versus chasmogamous (Silvertown, 1984; Kigel, 1995). For these species, seeds with high dispersal potential are less dormant than seeds with low dispersal potential (Venable and Lawlor, 1980; Cheplick, 1996a; Olivieri, 2002). Seed dispersal is more effective when seed weight is low (Westoby *et al*, 1996; Rees, 1997), but larger seeds produce seedlings that are more competitive and they have a better chance of surviving (McNamara and Quinn, 1977; Cheplick and Quinn, 1982; Cheplick, 1994; Cheplick and Wickstrom, 1999).

Punagrass and flechilla grande exhibit a heteromorphic seed pattern associated with cleistogamy. Both species have aerial chasmogamous (CH) seeds from apical panicles and cleistogamous (CL) seeds on axillary panicles that are enclosed within leaf sheaths (Cano and Eilberg, 1969; Eilberg, 1974; Cano, 1988). In the case of punagrass, the CL seeds are of different shape and size, and they develop in all the nodes of the stem. In flechilla grande, 2 or 3 CL seeds develop only at the base of the plant near the ground level (Cano and Eilberg, 1969; Eilberg, 1974; Cano, 1988).

It was hypothesized that CL and CH seeds have different dormancy mechanisms related to dispersal and seedling competition. The dormancy mechanisms may also differ between punagrass and flechilla grande, which can explain, in part, the success of punagrass as an invasive species on disturbed, fertile grasslands in Argentina. The general purpose of this study was to examine the germinability of the more dispersible CH and the less dispersible CL seeds of two species that exhibit seed heteromorphism associated with cleistogamy but differ in terms of functional characteristics, competitive ability and palatability to herbivory. Specific objectives of this study were to determine: 1) dormancy breaking and germination of CH and CL seeds of punagrass and flechilla grande; 2) the relationship between seed size and germination rate; and 3) the effect of range condition on seed size, germination and seed viability of CH and CL seeds in punagrass.

3.2 Materials and Methods

3.2.1 Study sites

Seeds were collected from the dry caldén forest in the semiarid region of the Province of La Pampa, Central Argentina. Seeds of punagrass were obtained from two areas with contrasting range conditions. One area was moderately grazed and it was in good range condition (GC), with more than 50% cover from palatable grasses. The other area had been subject to heavy grazing and was in poor range condition (PC) with less than 25% cover of palatable grass. Seeds of flechilla grande were collected only from the PC grassland.

The GC grassland is located at “Puesto Colorado” (36°12'S, 65°00'W), and the PC grassland is at “Bajo Verde” (36°28'S, 64°36'W). The landscape of the GC grassland is characterized by gently rolling terrain surrounded by hills and sandy accumulations while the PC grassland is a depression surrounded by upland plains (INTA *et al*, 1980). The GC grassland is under the open canopy of mature caldén (*P. caldenia*) (> 5 m in height), caldén saplings (< 5 m in height) and shrubs. The herbaceous layer is composed of more than 50% cover of palatable, cool-season, short grasses (*P. ligularis; P. napostaense*), and the rest are unpalatable mid-height cool-season grasses (*A. brachychaetum; S. tenussima; Stipa trichotoma* Nees*; S. gynerioides*), warm-season

grasses (*D. californica*), and forbs. Vegetation of the PC grassland is similar to the GC grassland but with more than 50% cover of unpalatable, cool-season grasses.

The climate of both sites is semiarid with a mean July temperature of 8 °C, and a mean January temperature of 24°C. Annual precipitation is 550-660 mm and the water deficit is approximately of 250-300 mm annually (INTA *et al* 1980). Soils in the GC grassland are Entic haplustols with sandy to silty clay loam texture, well drained with moderate organic matter content. Soils in the PC grassland are Mollisols, with silty clay loam texture, and moderate organic mater content (INTA, 1980).

The GC grassland was used for sheep grazing during the first half of the 20th century, which was later replaced with cattle grazing. The grassland is currently grazed during fall and winter. Before cool-season grasses start flowering, the herd is moved to pastures. Two accidental summer fires were recorded in the 1960s and the 1980s, respectively by wood scar analysis in a neighbouring ranch (Dussart *et al*, 1998). Periodic, controlled fires are used to enhance grass regeneration. The PC grassland had a similar change in the management from sheep to cattle grazing, and it is continuously grazed. Three accidental summer fires have been recorded between 1995 and 2001.

3.2.2 Seed collection

CH seeds of punagrass were collected in December 2002 (early summer) and CL seeds in March 2003 (late summer). CH and CL seed collection of flechilla grande was made in the early summer of 2001. Seeds were obtained from maternal plants of similar size. Seeds of punagrass were collected in four homogeneous, randomly selected sites that had high densities of adult plants at GC and PC grasslands. Seeds of flechilla grande were collected from plants in three homogeneous, randomly selected sites at PC grassland. Seeds were pooled within each site according to node and mating type (CH or CL; CL seeds from the 1^{st} to 7^{th} node along the tiller). Seeds of punagrass were air dried and stored in paper bags at room temperature in the laboratory until use. Seeds of flechilla grande were air dried and stored in paper bags in darkness at room temperature in the laboratory for three weeks, and then at -18° until use.

3.2.3 Data collection

3.2.3.1 Effect of dormancy breaking treatments on germination of chasmogamous and cleistogamous seeds from different node positions of punagrass

A completely randomized design (CRD) with four replications was used for germination tests and there were 25 seeds per experimental unit. CH and CL seeds from the 1st to the 5th node were used. For CH and CL seeds, treatments were control and prechilling. Additional treatments included control hulled and dehulled for CH seeds and control and scarification for CL seeds.

Seeds used for the experiment came from the PC grassland. At the time of experiments, CH seeds had 9 months, and CL seeds 6 months of dry after-ripening. Individual seeds were weighed using a Microbalance (Cahn C-33). CH seeds were weighed with and without palea and lemma. Empty seeds and immature seeds with palea and lemma that weighed less than 0.8 mg per seed were discarded.

One-half of the CH seeds were dehulled (palea and lemma removed) and half of the CL seeds were scarified with sand paper on the side opposite of the embryo. Prechilling at 4 °C for two weeks was applied to one-half of the CH and CL seeds. Seeds were arranged in a grid into the Petri dishes containing water-saturated filter paper, and each seed was placed in a marked cell. Petri dishes were put in closed containers to avoid moisture loss and wrapped in black plastic bags. Seeds were incubated in a growth chamber (Conviron) at 25/15 °C, 9/15 h in darkness.

Seeds were considered germinated when the radicle was at least 2 mm long. Germination data were recorded every 2 days for 28 days and then periodically up to 120 days. A green safe light was used during the germination check.

3.2.3.2 Effect of dormancy breaking treatments on germination of chasmogamous and cleistogamous seeds of flechilla grande

A CRD with four replications was used for germination tests and there were 25 seeds per experimental unit. CH and CL seeds were used. The treatments were hulled, dehulled, darkness and light for both seed types.

At the time of experiments flechilla grande seeds had 10 months of dry after-ripening. Individual seeds were weighed using a Microbalance (Cahn C-33). CH and CL

seeds were weighed with and without palea and lemma. Awns were removed in CH seeds before weighing. Empty seeds and immature seeds with palea and lemma that weighed less than 5 mg per seed were discarded. The palea and lemma were removed for one-half of the CH and CL seeds. One-half of the Petri dishes were wrapped in black plastic bags. Seeds were incubated in a growth chamber (Conviron) at 25/15 °C, 9/15 h (Cabeza *et al*, 1999) with 9-h light (400 Lux of white cool fluorescent) and 15-h darkness (light treatment) or with 24-h darkness (darkness treatment).Otherwise, similar procedures in 3.2.3.1 were followed.

3.2.3.3 Effect of range condition on seed mass and germinability of chasmogamous and cleistogamous seeds of punagrass

A CRD with four replications per seed type (CH or CL from the 1st to 6th node) and range condition type (GC or PC) was used for germination tests and there were 50 seeds per experimental unit. Three field locations within each area were used as replications. The treatments were GC CH, GC CL from the 1st to 6th node, PC CH, and PC CL from the 1st to 6th node. A CRD with three replications per seed type (CH or CL from the 1st to 6th node) and range condition type (GC or PC), with 20 seeds per experimental unit was used for seed viability tests.

Seeds used for the experiments came from the PC and GC grasslands. Three randomly selected sites of good or poor range condition were used because seeds were not available for one of the PC sites. At the time of germination tests, CH seeds had 19 months and CL seeds 16 months of dry, after-ripening. Individual CH and CL seeds from 7 node positions were weighed using a Microbalance (Cahn C-33). About 400 seeds per node position and range condition were weighed. CH seeds were weighed with the palea and lemma intact. Empty seeds and immature seeds that weighed less than 0.8 mg per seed were discarded.

Palea and lemma of CH seeds were removed before incubation. Seeds were incubated in a growth chamber (Conviron) at 20/10 °C, 9/15 h in darkness (Eilberg and Soriano, 1972). Germination data was recorded weekly during 28 days. Ungerminated seeds were tested for viability with a 0.1% solution of 2, 3, 5-triphenyl-tetrazolium chloride (Grabe, 1970). Seeds were bisected longitudinally and half of the seeds were soaked in the solution and incubated at room temperature for 24 h. Seeds were

considered viable when the whole embryo was stained. Evaluation was done under a magnifier glass. Otherwise, similar procedures in 3.2.3.1 were followed.

3.2.4 Data Analysis

The effect of treatments on final germination percentage of seeds across node positions or flower type was analyzed using a factorial analysis of variance (ANOVA) (Steel and Torrie, 1997). Treatment, flower type or node position and their interactions were the sources of variance. The effect of the dehulling treatment on final germination percentage of CH seeds was analysed using an one-way ANOVA. The effect of range condition on final germination percentage, individual seed mass and viability across tiller node positions or flower type was evaluated using a factorial ANOVA. Range condition, node position and their interactions were the sources of variance. Additional one-way ANOVAs were performed within flower type or node position. To meet ANOVA assumptions, percentage data were arcsine square –root transformed (Underwood, 1997). Fisher' least significant difference (LSD) tests were performed for mean separation with a significant level at P = 0.05 (Steel and Torrie, 1997). All the analyses were performed with GLM of SAS system (SAS Institute Inc., Release 8.02, Cary, NC, USA).

3.3 Results

3.3.1 Effects of dormancy breaking treatments on germination of chasmogamous and cleistogamous seeds from different node positions of punagrass

The interaction between node position and prechilling treatment on final germination was significant (P = 0.004). Prechilling reduced germination of seeds from the 1st node (P = 0.004) but the prechilling did not affect germination of other seed types when analyzed within node positions (Fig. 3.1). Germination of CH seeds was not affected by the dehulling treatment with an average germination percentage of 25% ± SE 4%. Germination of CL seeds was not affected by scarification.

Final germination was low with or without dormancy breaking treatments (Fig. 3.1). CH seeds had greater germination than CL seeds.

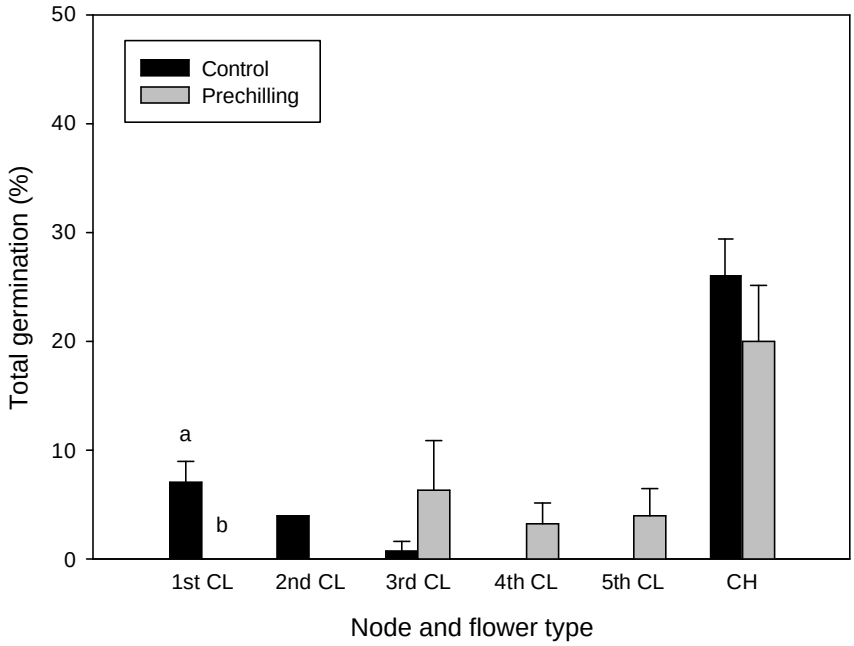

Figure 3.1 Effect of prechilling on germinability of chasmogamous (CH) and cleistogamous (CL) seeds at different node positions in punagrass (*Achnatherum brachychaetum*). Seeds were collected from grassland in poor range condition. CH and CL seeds were dry-stored for 9 and 6 months, respectively and incubated at 25/15° C, 9-h/15-h in darkness. The prechilling treatment was conducted for 2 weeks at 4°C. Values are Means ± SE. Different lower case letters within each seed type denote significant differences among treatments (P = 0.05).

For the non-dormant fraction of the seed population, germination rate or time to germination varied among seed types and with seed mass (Fig. 3.2). Time to germination tended to be shorter for the smaller CH seeds as compared to the larger CL seeds.

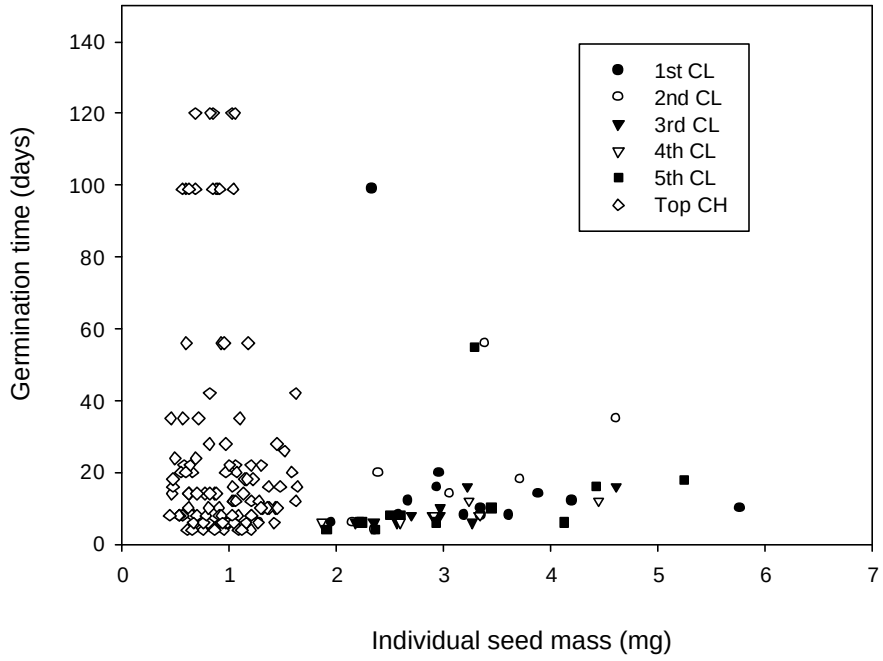

Figure 3.2 Relationship between germination time and individual seed mass of chasmogamous (CH) and cleistogamous (CL) seeds in punagrass (*Achnatherum brachychaetum*). Seeds were collected from grassland in poor range condition and incubated at 25/15° C, 9-h/15-h in darkness.

3.3.2 Effect of dormancy breaking treatments on germination of chasmogamous and cleistogamous seeds of flechilla grande

In flechilla grande, final germination was not affected by seed type or light treatment, but it was increased by removing the palea and lemma ($P < 0.001$) (Fig. 3.3). The treatment by seed type interaction was not significant. Germination was enhanced by dehulling, increasing from 23 to 94% for CL seeds and from 43 to 81% for CH seeds.

Most of the non-dormant seeds of flechilla grande germinated in less than 2 weeks, faster than Punagrass (Figs 3.2, 3.4). While individual seed mass tended to decrease from bottom to top along the tiller, a relationship between germination rate (time to germination) and seed mass was not apparent.

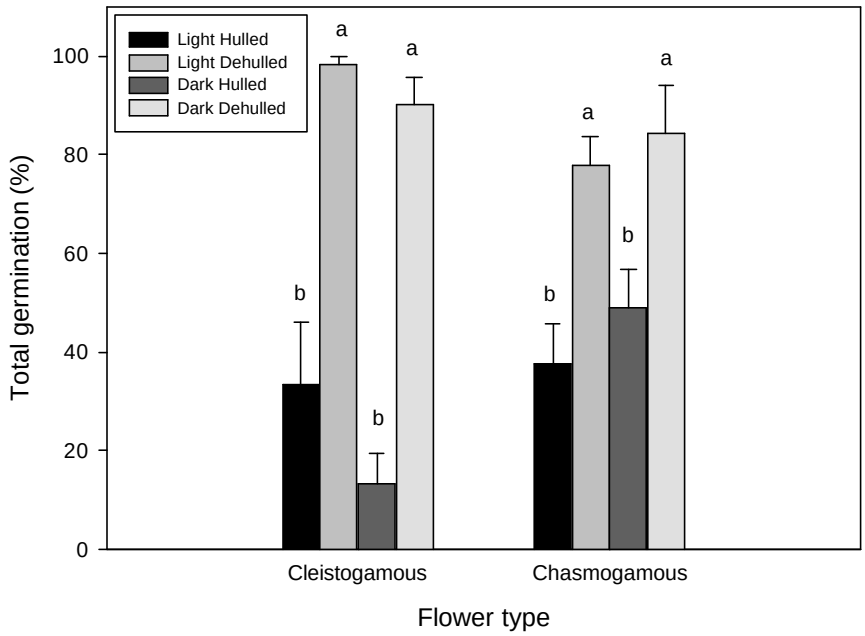

Figure 3.3 Effect of light and dehulling treatments on germination of chasmogamous and cleistogamous seeds in flechilla grande *(Nassella clarazii*). Seeds were collected from grassland in poor range condition. Seeds were dry-stored for 10 months, and incubated at 25/15° C with 9-h light/15-h darkness or 24-h darkness. Values are Means ± SE. Different lower case letters within each seed type denote significant differences among treatments (P = 0.05).

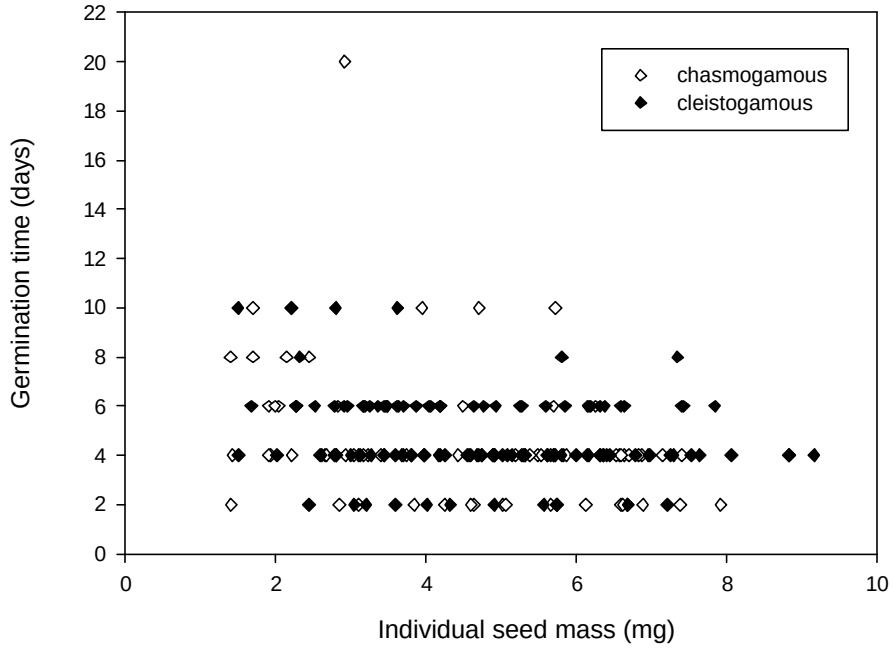

Figure 3.4 Relationship between germination time and individual seed mass of chasmogamous and cleistogamous seeds in flechilla grande *(Nassella clarazii*). Seeds were collected from grassland in poor range condition and incubated at 25/15° C with 9-h light/15-h darkness or with 24-h darkness. Seed mass was determined without the palea and lemma.

3.3.3 Effect of range condition on seed mass, germination and viability of chasmogamous and cleistogamous seeds of punagrass

Seed mass decreased from the bottom to top of the tillers in punagrass (Fig. 3.5). The interaction between seed type and range condition was significant (P = 0.020) indicating that the effect of range condition on mass varied among seed positions or mating mechanisms (CH or CL). The interaction between seed type and range condition was not significant when CH seeds were not included in the analysis. CL seeds from good range condition grassland were heavier than those from poor range condition grassland (P<0.001). The effect of range condition on CH seed mass was not significant.

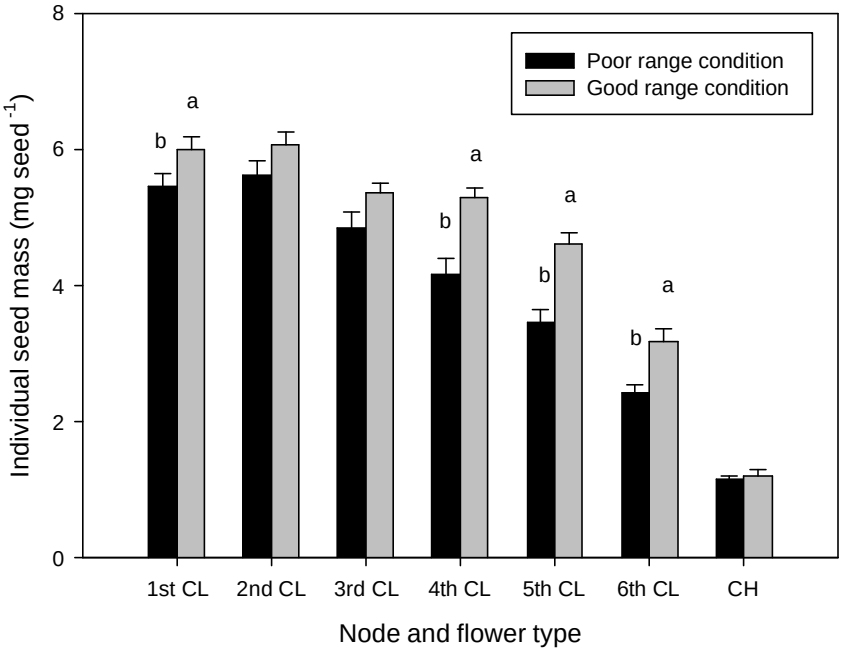

Figure 3.5 Effect of range condition on the individual seed mass of chasmogamous (CH) and cleistogamous (CL) seeds at different node positions in punagrass (*Achnatherum brachychaetum*). Seeds were collected from grasslands in poor and good condition. Values are Means ± SE. Different lower case letters within each seed type denote significant differences among range conditions (P = 0.05).

Final germination was affected by the interaction between seed type and range condition (P = 0.007), suggesting that germination of CH and CL seeds responded differently to range condition (Fig. 3.6). Germination of CH seeds was higher in areas with good range condition than poor condition, averaging 60 and 52%, respectively when analyzed within seed type (P = 0.035). Total germination of CL seeds was mostly

less than 5%, indicating high dormancy after 16 months of dry after- ripening. Germination of CL seeds from the 5th node were affected by range condition (P = 0.039), with higher germination in the poor range condition than the good range condition.

Although differences in seed viability among seed types were not significant, they were different between conditions (P = 0.033) (Fig. 3.7). There was no significant interaction between range condition and node. Viability of seeds from poor range condition sites was 15% lower than from good condition range.

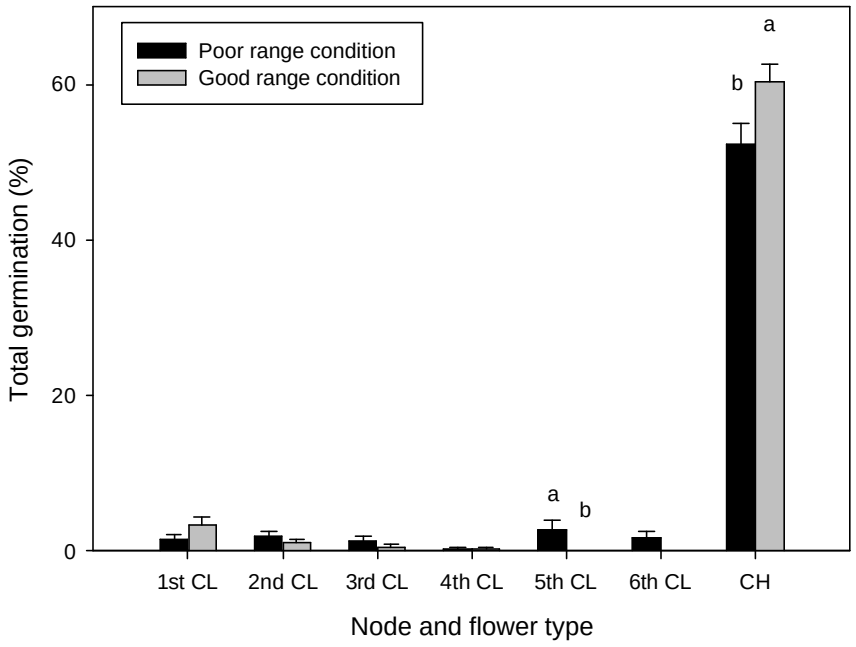

Figure 3.6 Effect of range condition on the germination of chasmogamous (CH) and cleistogamous (CL) seeds at different node positions in punagrass (*Achnatherum brachychaetum*). Seeds were collected from grasslands in poor and good condition. CH and CL seeds were dry-stored for 19 and 16 months, respectively and incubated at 20/10° C, 9-h/15-h in darkness. Values are Means ± SE. Different lower case letters within each seed type denote significant differences among range conditions (P = 0.05).

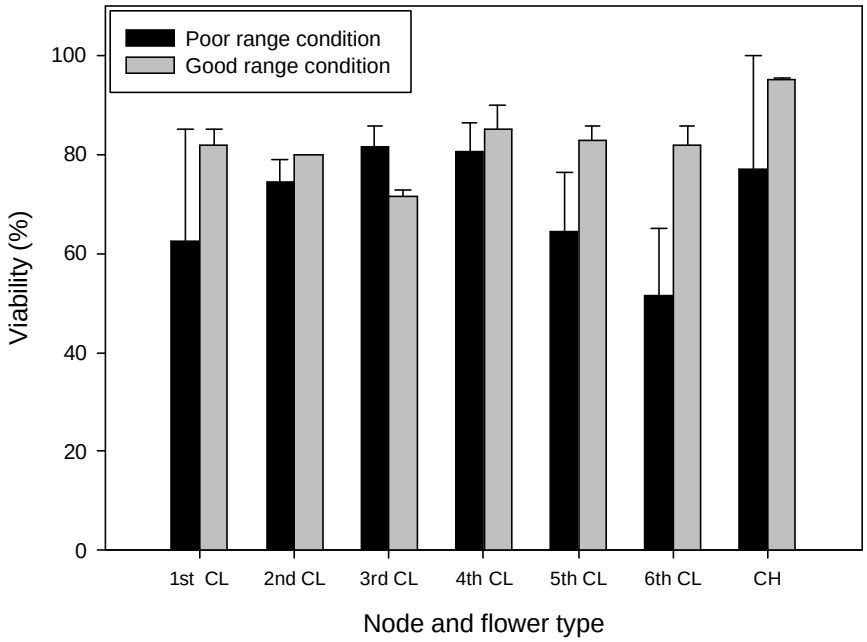

Figure 3.7 Effect of range condition on the viability of seeds derived from chasmogamous (CH) and cleistogamous (CL) seeds at different node positions in punagrass (*Achnatherum brachychaetum*). Seeds were collected from grasslands in poor and good condition. CH and CL seeds were dry-stored for 19 and 16 months, respectively. Values are Means ± SE. Different lower case letters within each seed type denote significant differences among range conditions (P = 0.05).

3.4 Discussion

3.4.1 Ecological significance of contrasting dormancy patterns between chasmogamous and cleistogamous seeds and between Punagrass and Flechilla grande.

Dormancy was high in CH and CL seeds of punagrass and removal of palea and lemma, stratification or scarification were not effective in breaking dormancy. Germination averaged 25% for CH seeds and less than 3% for CL seeds. A 40% germination of CH seeds and 10% germination of CL seeds were reported for punagrass when seeds were placed on the soil surface after harvest in the fall (Eilberg and Soriano, 1972). A study on punagrass in pastures of alfalfa showed that most seedlings originated from CL seeds (Ares *et al*, 1970b). High dormancy in CL seeds of punagrass may enable them to form a persistent seed bank in the soil.

In many species with seed heteromorphism, such as *S. vaginiflorus* and *T. purpurea* (Cheplick 1996a) total germination and germination rate are different between seeds with high and low dispersal potentials (Venable and Lawlor, 1980; Cheplick, 1998). Seed dispersal is more effective when seed weight is low as dispersed seeds are packed with lighter energy sources (Westoby *et al*, 1996; Rees, 1997). Furthermore, seeds with low dispersal potential usually have greater dormancy than seeds with high dispersal potential within a species (Venable and Lawlor, 1980; Cheplick, 1996a; Olivieri, 2002). A model for the effect of seed dispersal and inbreeding on the evolution of seed dormancy also predicts that dormancy increases as the dispersal potential of seeds decreases, and inbreeding delays germination to avoid sibling competition for limited resources (Kobayashi and Yamamura, 2000). Sibling competition due to restricted dispersal of CL seeds reduced growth more than competition between unrelated plants, in accordance with the resource-partitioning hypothesis (Cheplick, 2004). On the other hand, outcrossing breeding systems may be selected to minimize sibling competition. Therefore, large, dormant CL seeds of punagrass have probably ecological importance in terms of persistence in the soil seed bank and avoidance of intraspecific competition. CH seeds of punagrass are more likely well-dispersed and have greater and faster germination than the CL seeds. More diverse offspring may have greater ability for the partition of limiting resources.

Germination of CH and CL seeds of flechilla grande was higher than punagrass, and most dormancy was broken by the removal of palea and lemma. A 97 % germination of CH seeds was reported for flechilla grande after similar treatments (Cabeza *et al*, 1999). The lemma and palea can markedly constrain seed germination of grasses (Simpson, 1990). CH and CL seeds also germinated better with scarification of lemma and palea in *Danthonia californica* (Laude, 1949).

Most studies on seed germination of species with CH and CL seeds, including punagrass, show that the two types of seeds behave differently (Campbell *et al*, 1983). However, germination in flechilla grande was similar between seed types. Species with similar germination patterns between CH and CL seeds include *Danthonia unispicata* (Thurb.) Munro ex Macoun. (Dobrenz and Beetle, 1966) and *D. californica* (Laude, 1949; Dobrenz and Beetle, 1966). Nevertheless, seedlings from CH seeds of *D.*

californica had greater emergence percentage than CL seeds under a variety of field conditions (Laude, 1949). Although both seed types of flechilla grande displayed similar germination patterns at optimum conditions in the laboratory, differences in germination may occur in the field.

Unlike Punagrass, CH and CL seeds of flechilla grande did not consistently differ in seed size. The perennial grass, *D. spicata,* had no differences in seed mass between CH and CL seeds, but greater differences were found at the juvenile stage than the adult stage between CH and CL progeny (Clay, 1983b; Clay and Antonovics, 1985). CH and CL seeds of the perennial grass *D. clandestinum* are similar in size, but the percentage, rate of germination and percentage of seedling emergence from seed types both in the field and in greenhouses are higher for CL progeny (Bell and Quinn, 1985). When CH and CL progeny were grown in mixture at the low densities, CH progeny were heavier and had more spikelets than CL progeny. Competition for resources among CH progeny was not as high as among CL progeny in *D. clandestinum* (Bell and Quinn, 1985). One advantage of having CH and CL seeds in flechilla grande can be to have more variable progeny, reducing sibling competition at the juvenile stage.

For most species that produce CL seeds at the base of the plant, seed differences in terms of specialized dispersal mechanism is typical (Campbell *et al*, 1983). The CH seeds of flechilla grande have a hygroscopic awn (Cano, 1988), which can have a significant role in seed burial (Garnier and Dajoz, 2001). CH seeds of flechilla grande with mechanisms for delayed germination (physical dormancy), dispersal and/or burial, as well as limited seed production may have ecological significance for persisting in the few safe sites in semiarid areas, while minimizing intraspecific and interspecific competition.

3.4.2 Grazing as a short-term selective force on seed mass and germinability of punagrass

Different patterns of seed heteromorphism associated to CL seeds were found in populations of punagrass from areas with contrasting range condition. Individual mass of CL seeds was higher in GC grasslands with higher competition from palatable grasses than in overgrazed, PC grasslands. Seed size is positively correlated with seedling emergence, establishment and competitive ability (Rees, 1997; Leishman *et al*, 2000;

Leishman, 2001). Larger CL seeds produce seedlings that are more competitive and have better chances of surviving (McNamara and Quinn, 1977; Cheplick and Quinn, 1982; Cheplick, 1994; Cheplick and Wickstrom, 1999). When competition is severe, natural selection favours seeds with large energy reserves, which in turn often limits dispersal (Willson and Traveset, 2000). It is suggested that under competitive conditions, the priority for Punagrass would be to increase seed size and competitive ability of seedlings.

Large seeds permit seedling persistence under the relatively unfavourable conditions associated with closed cover of vegetation at later successional stages (Burke and Grime, 1996). Large seeds confer improved tolerance against a wide variety of hazards, as seedlings of large-seeded species tend to have more reserves available to compensate for adverse conditions (Leishman *et al*, 2000). The persistence may provide the possibility of subsequent opportunistic responses to resource pulses arising from local mortalities in established, neighbouring plants (Campbell and Grime, 1989). Large CL seeds of Punagrass can be advantageous for persistence as seedlings in competitive communities, such as areas in good range condition, until the creation of gaps with low competitive pressure.

Dormancy of CL seeds was high and similar in areas with contrasting range condition. Range condition or site history also affected seed germination with CH seeds from poor condition area being 8% lower than that from good condition area. These differences may be due to seed quality, as a 15% reduction in seed viability was found in the PC areas.

3.5 Summary

Seed heteromorphism in punagrass was expressed in marked differences in size and germination between CH and CL seeds. Seed heteromorphism in flechilla grande was not consistently expressed in size and dormancy as CH and CL seeds had similar mass, germination, and response to dormancy breaking treatments. CH seeds were larger in flechilla grande than in punagrass, which reflects differences in colonization/competition strategies between the two species. Small CH seeds may confer high capacity for colonizing disturbed habitats for punagrass, and large CL seeds may give high ability for competition for flechilla grande.

In agreement with theoretical and experimental data, seeds of low dispersal potential were more dormant than seeds with high dispersal potential in punagrass. The dormancy of the CL seeds and the more diverse CH seeds can reduce sibling competition. Production of CL seeds and the more variable CH seeds in flechilla grande are probably adaptations for partitioning limiting resources in semi-arid grasslands of central Argentina.

Punagrass had bigger seeds in areas of good range condition than in overgrazed areas with reduced competition from palatable grasses. Larger CL seeds of punagrass are expected to produce seedlings with greater competitive ability and to persist in the soil seed bank. In areas dominated by palatable grasses, gaps of low competitive pressure may favour growth and development of punagrass.

4. RELATIVE CONTRIBUTION OF CHASMOGAMOUS AND CLEISTOGAMOUS SEEDS TO THE FITNESS OF PUNAGRASS AND FLECHILLA GRANDE

4.1 Introduction

Species with seed heteromorphism can have distinct seed types maturing at different positions on the maternal plant often differing in their mass and the number produced (Silvertown, 1984; Venable, 1985; Cheplick and Clay, 1989; Kigel, 1995; Cheplick, 1996b, Gutterman, 2000). In many grasses, cleistogamous (CL) seeds are produced at different nodes while chasmogamous (CH) seeds are produced on terminal panicles (Campbell *et al*, 1983; Cheplick and Clay, 1989; Cheplick, 1996b).

In species with mixed mating systems bearing both CH and CL flowers, floral or seed type may have a large impact on the fitness of progeny (McNamara and Quinn, 1977; Cheplick and Quinn, 1982; Clay, 1983b). For example, seedlings from CL seeds are larger and have higher survival than those from CH seeds in many species (McNamara and Quinn, 1977; Cheplick and Quinn, 1982; Cheplick, 1994; Cheplick and Wickstrom, 1999). Plants from CH seeds grew less, produced fewer seeds, and allocated less resources to reproduction than plants from CL seeds (Cheplick and Quinn, 1982). Plants from CL seeds flowered sooner than those from CH seeds (McNamara and Quinn, 1977; Cheplick and Quinn, 1982). Mixed mating systems may be selected for as a way to minimize sibling competition. More diverse offspring relative to similar offspring compete less intensely because of greater partition of limited resources (Cheplick, 2004). The growth of plants from CH seeds was severely depressed in mixtures of plants from the two types of seeds at two densities in *A. purshii* (Cheplick and Quinn, 1983). In the perennial grass *D. clandestine*, the CH progeny had greater

total shoot biomass, number of spikelets per plant and was more competitive than the CL progeny at low-density mixture of CH and CL plants (Bell and Quinn, 1985).

The relative proportion of CH to CL flowers (and seeds) in plants can vary greatly among populations of perennial grasses (Clay, 1983a; Bell and Quinn, 1987). This can be due to changes in the ratio of CH to CL seeds as succession progresses (McNamara and Quinn, 1977). Moreover, abiotic and biotic stress can result in a shift in the mating system toward greater production of CL flowers (and seeds) (Bell and Quinn, 1987; Le Corff, 1993; Cheplick, 1994; Bennington and McGraw, 1995; Culley, 2002; Steets and Ashman, 2004).

Plants derived from CH and CL seeds may have different growth and reproductive characteristics. Species with different functional characteristics, competitive ability and palatability to herbivory such as punagrass and flechilla grande may have different mechanisms regulating reproduction and fitness. The objectives of this study were to determine: 1) the influence of seeds produced chasmogamously and cleistogamously on reproductive phenology, developmental morphology, growth, seed production, seed heteromorphism and reproductive allocation of their progeny in punagrass and flechilla grande, and 2) the effect of range condition on reproductive phenology, developmental morphology, growth, seed production, seed heteromorphism and reproductive allocation of CH progeny in punagrass.

4.2 Materials and Methods

4.2.1 Study sites

Seeds were collected from the dry caldén forest in the semiarid region of Province of La Pampa, Central Argentina. Seeds of punagrass were obtained from two areas with contrasting range conditions. One area was properly grazed and in good range condition (GC) with more than 50 % cover with palatable grasses; the other area had been overgrazed and was in poor range condition (PC) with less than 25 % palatable grass cover. Seeds of flechilla grande were only collected from the PC grassland. For more details about study sites see Chapter 3 (3.2.1).

4.2.2 Seed collection

CH seeds of punagrass were collected in December 2002 (early summer) and CL seeds in March 2003 (late summer). CH and CL seed collection of flechilla grande was made in the early summer of 2001. Seeds were obtained from maternal plants of similar size. Seeds of punagrass were collected in four randomly selected, homogeneous sites that had high densities of adult plants within GC and PC areas. Seeds of flechilla grande were collected from three randomly selected, homogeneous sites in the PC area. Seeds were pooled within each site according to node and mating type (CH or CL for flechilla grande; CH and CL seeds from the 1^{st} to 7^{th} node along the tiller for puna grass). Hulls of CL seeds of punagrass were removed by hand. Seeds of punagrass were air dried and stored in paper bags at room temperature in the laboratory until use. Seeds of flechilla grande were air dried and stored in paper bags in darkness at room temperature in the laboratory for three weeks, and then at -18° until use.

4.2.3 Experimental design

For the first Experiment, seeds from PC area were used for both species. For punagrass, seedlings derived from CH seeds and CL seeds matured at the 1^{st}, 3^{rd} and 5^{th} nodes on the tiller were used due to low number of surviving seedlings from the rest of the nodes. For flechilla grande, seedlings derived from CH and CL seeds were used. A randomized complete block design (RCBD) with four replications was used. There were two subsamples per replication.

For the second Experiment, only CH seeds of punagrass were used due to unsuccessful transplants of seedlings from CL seeds of the GC grassland. CH seeds from three sites in each area (GC or PC) were used. A RCBD with four replications was used and there were two subsamples per replication.

4.2.4 Plant growth conditions

Seedlings of 4-6 days old and similar height were randomly selected from previous germination experiments (Chapter 3) and planted in potting soil (Terra Lite Redi Earth) in plug flats (48 cells, 9 cm^2 / flat TLC Polyform). Seedlings were watered daily to field capacity and remained in the growth chamber for two weeks at 25/15° C (9/15 h), 9-h light (400 Lux of white cool fluorescent)/15-h darkness. Trays with water

were placed in the growth chamber to maintain moisture. Seedlings were then transferred to pots (27 cm in diameter x 23 cm in depth) containing the same potting soil in a greenhouse. Soil analysis were performed (n=5) to determine the concentration of nitrates (Autoanalyzer Colorymeter, Pulse Instrumentation Ltd., 10:1 water extraction) and phosphorus (Kalowna Method). The soil used contained 80 ppm of Nitrogen and 35 ppm of Phosphorus. After 4 weeks of transplant, plants were fertilized with 20 ppm N and 90 ppm P, the level of nutrients similar to that found in the Argentine grasslands (Castelli and Lazzari, 2002). Plants were watered twice a week using tap water during growth.

4.2.5 Data collection

For Experiment 1, plant height, total leaf number, and tiller number per plant at the time of the fertilization [35 days after planting (DAP)] were recorded for both species. These parameters were measured again 76, 90 and 118 DAP. The following stages of reproductive phenology, associated to CH flowers and seeds, were also recorded 90, 99, 118, 129 and 141 DAP for punagrass: stem elongation (3 nodes visible), boot stage, first spikelet visible, spikelet fully emerged/peduncle not emerged, inflorescence emerged/peduncle fully elongated, anther emergence/anthesis, developing seed/caryopsis visible, and seed ripe. The total number of tillers per plant at each stage and date was recorded. The total number of tillers producing CH seeds in terminal panicles were counted at harvest. Since plants of flechilla grande did not flower under greenhouse conditions, after 100 DAP they were placed in growth chambers at 15/10°C, light-dark (8/16 h) for two weeks, followed by 10/6°C light-dark (8/16 h) for another two weeks, and again at 15/10°C, light-darkness (8/16 h) for one week. Plants were transferred back to the greenhouse after the treatments. At harvest time, the number of tillers per plant at each of the following phenological stages were evaluated: stem elongation (3 nodes visible), boot stage, developing seed/caryopsis visible and seed ripe.

Since CH seeds matured early, terminal panicles in punagrass were harvested first, and the remaining tillers were labelled and plants were given additional time for CL seeds to mature. All punagrass plants were harvested after 175 DAP, and after 197 DAP in flechilla grande. Soil was washed off the roots and entire plants were placed into paper bags. Up to 5 tillers that produced CH seeds in terminal emergent panicles (mature

tillers) were sampled per plant for seed production evaluation for punagrass. Another 5 tillers without terminal panicles were selected (young tillers) (Experiment 1 only). For flechilla grande, up to 5 tillers per plant were sampled. Once seeds were separated from the stems, plant materials were oven-dried to constant mass at 85°C. The dry mass of each tiller was recorded separately. Roots were also oven-dried to constant mass under the same conditions.

Vegetative development was determined at 29 and 69 DAP, and reproductive phenology at 69, 76 and 91 DAP for the plants of Experiment 2. Otherwise, similar procedures in experiment 1 were followed.

Tillers of punagrass were separated by nodes and examined for the occurrence of CL seeds. Seed number and total fresh seed mass per tiller were recorded for each node, including CH seeds. For flechilla grande, seed number and total fresh mass per tiller of CH and CL seeds were recorded. CL seeds were classified into fully developed, green and immature whereas that CH seeds into fully developed and immature. After hand cleaning, the fresh mass of fully developed CH, immature CH and fully developed CL seeds was measured using a Microbalance (Cahn C-33). CH seeds of both species and CL seeds of flechilla grande were weighed with hulls (palea and lemma). Hulls of CL propagules of punagrass were removed at harvest.

Seed germination was tested two months after harvesting for seeds from Experiment 1. Both CH and CL seeds of punagrass, but only CH seeds of flechilla grande were tested due to the shortage of CL seeds in the latter. Seeds of punagrass were tested in darkness at 20/10 °C, simulating fall conditions (Eilberg and Soriano, 1972), and at 25/15 °C and 30/20 °C, 9/15 h simulating spring and summer conditions in the field. Seeds of flechilla grande were incubated at 25/15 °C, 9/15 h in darkness (Cabeza *et al*, 1999). Seeds were separated according to progeny type and node position and placed in Petri dishes containing water-saturated filter paper. Petri dishes were put in closed containers to avoid moisture loss. The lemma and palea of CH seeds were removed for punagrass but not in flechilla grande. In punagrass, a split-plot design with four replications was used with temperature regime as main plot and subplots with four progenies types (seeds from plants derived from CH seeds or CL seeds produced at the 1^{st}, 3^{rd} and 5^{th} nodes on the tiller), six grand-progeny seed types (CH and CL seeds from

nodes 1st to 5th), and 25 seeds per experimental unit. In flechilla grande, a Completely Randomized Design (CRD) with four replications was used with two progeny treatments (CH seeds from plants derived from CH or CL seeds) and 25 seeds per experimental unit. Seeds were checked weekly for germination for 4 weeks.

Another germination experiment was carried out using seeds from Experiment 2. Seeds were incubated at 20/10 °C, 9/15 h in darkness. A CRD was used with four replications, two range condition classes and three seed types (CH seeds, and CL seeds of the 1st and 5th nodes). Seeds were checked weekly for germination for 4 weeks.

4.2.6 Data Analysis

Developmental morphology was quantified with a modified Moore numerical index system (Moore and Moser, 1995). This index can be used for studying developmental morphology in perennial grasses and is based on the ontogeny of individual tillers. Numerical values were assigned to the following growth stages: V (vegetative stage) =1.0, Eo (Onset of stem elongation) = 2.0, Ro (Boot stage) = 3.0, R1 (Inflorescence emergence/first spikelet visible) = 3.1, R2 (Spikelet fully emerged/peduncle not emerged) = 3.3, R3 (Inflorescence emerged/peduncle fully elongated) = 3.5, R4 (Anther emergence/anthesis) = 3.7, So (developing seed/caryopsis visible) = 4.0, and S5 (Seed ripe) = 4.9.

The mean developmental stage was calculated using the following equation:

$$MSC = \frac{\sum_{i=0}^{Max} \sum_{j=1}^{N_i} S_{ij}}{C} \quad (Eq.\ 4.1)$$

where MSC = mean stage count, Si = growth stage, i = 0 to maximum growth stage, Ni = number of tillers in stage Si, and C = total numbers of tillers.

The Relative Growth Rate (RGR) of height at early growth stage was calculated as (ln [plant height after 4 or 6 weeks] - ln [initial plant height at the first fertilization])/ 4 or 6 weeks.

Only fully developed CH and fully developed plus green CL seeds were included in seed production analysis. Seed number and seed mass per node and tiller represent the average seed production per node (including the CH seeds of the terminal panicle) of the sampled tillers that produced CH seeds. When the nodes did not have seeds, seed

number and mass were registered as zero and the average included zero values. The individual seed mass of each node was calculated using the total seed mass divided by the number of seeds per node and tiller. The final individual seed mass was the average of tiller nodes with seeds; nodes that lacked seeds were not taken account in the average. Total seed number, total CL seed number, total seed mass and total CL seed mass per tiller were calculated as the sum of the parameters in all the nodes of the tiller, and then averaged for all sampled tillers. Total CH seed number per plant was calculated as the average CH seeds per tiller * total number of mature tillers. CL seed number per plant of the four lowest nodes was calculated as the average seed number per node * total number tillers (mature plus young tillers). Seed number of the rest of the nodes was calculated as the average seed number per node * total number of mature tillers. Total CL seed number per plant was the sum of all CL seeds produced per node per plant. CH and CL seed mass per node per plant and total per plant were calculated in the same way as seed number. Total seed number and seed mass per plant were the sum of CH and CL seed number and mass per plant, respectively. Total tiller mass per plant was calculated as the (average tiller biomass of mature tillers * number of mature tillers) + (average tiller biomass of young tillers * number of young tillers).

Reproductive allocation per node per plant was calculated as the percentage of total seed mass in total plant biomass, which included total seed mass per plant + total tiller biomass+ root biomass. CH and CL reproductive allocation per plant was calculated as the percentage of CH and CL seed biomass per plant in total plant biomass. All plants were measured for vegetative and reproductive development and phenology, but only one subsample per replication was evaluated for seed production and reproductive allocation due to time limitation.

Parameters for vegetative development, reproductive phenology, seed production and reproductive allocation were analysed using GLM of SAS system (SAS Institute Inc., Release 8.02). GLM with Type III sum of squares were used to analyze the effect of progeny type (fixed effect) within each phenological stage, date, flower type or node position. Block was treated as a random effect. Prior to analysis, data were examined for normality and homocedasticity. The percentage of reproductive tillers per plant and reproductive allocation were arcsine square –root transformed to meet model

assumptions (Underwood, 1997). Fisher' least significant difference (LSD) tests were performed with a significance level at P = 0.05 (Steel and Torrie, 1997). For flechilla grande, the model included progeny and flower type as fixed effects and block as a random effect.

For punagrass, seed number, seed mass and individual seed mass were also analysed using node position as repeated measures on the experimental unit or subject (plant). These variables may be correlated across observations (positions) on the same subject (Schabenberger and Pierce, 2002). The Durbin-Watson statistic was used as a measure of serial correlation (SAS Institute Inc., Release 8.02). Positive autocorrelation across node positions in seed mass per node, seed number per node and individual seed mass was highly significant ($P < 0.001$) when CH plus CL seeds of all nodes were included. Correlations were also highly significant ($P < 0.001$) for seed number per node, and significant ($P < 0.05$) for seed mass per node and individual seed mass only considering CL seeds. Therefore, measurements of the variables across seed positions were considered repetitions on the experimental unit with correlated residuals. Repeated measures analysis were performed with GLM procedure, with node position as within-subject effect (repeated statement) and progeny type or site history type as between-subject effect. The orthogonal polynomial transformation option was selected to implement the analysis. This transformation allows studying the nature of a repeated measures effect, accounts for substantial portions of the error variance over the repeated measurements, and having heterogeneous correlations among residuals (Rowell and Walters, 1976; Gurevitch and Chester, 1986). GLM with Type I sum of squares were used to test the nth degree contrasts. The Type I F-tests or sequential sum of squares were used for performing backward selection process using only a model fit; higher order terms were considered first and then drop them from the model if they were not significant. Because the previous analysis did not show a significant block effect, the block factor was not included in the model.

The two germination experiments for punagrass cannot be statistically analyzed because germination was low and close to zero in most of the experimental units. For flechilla grande, germination data were analyzed with GLM in SAS system. The square root of germination data was arcsine-transformed before analysis to meet model

assumptions. Fishers' LSD test was performed for mean separation using a significance level at P = 0.05.

4.3 Results

4.3.1 Phenology, developmental morphology and growth of CH and CL progeny of different node positions

Plants of punagrass started developing CH flowers 90 DAP and by 99 DAP significant differences among progeny types in the number of tillers per plant at the stage Eo (onset of stem elongation) ($P < 0.001$) and R2 (spikelets fully emerged/peduncle not emerged) ($P = 0.040$) were detected (Fig. 4.1). CL progeny produced more tillers with CH flowers than CH progeny at the Eo stage. CL progeny from the 5th node had more tillers at the R2 stage than any other type of progeny. Progeny from the 3rd node had a wider range of developmental stages than any other types of progeny.

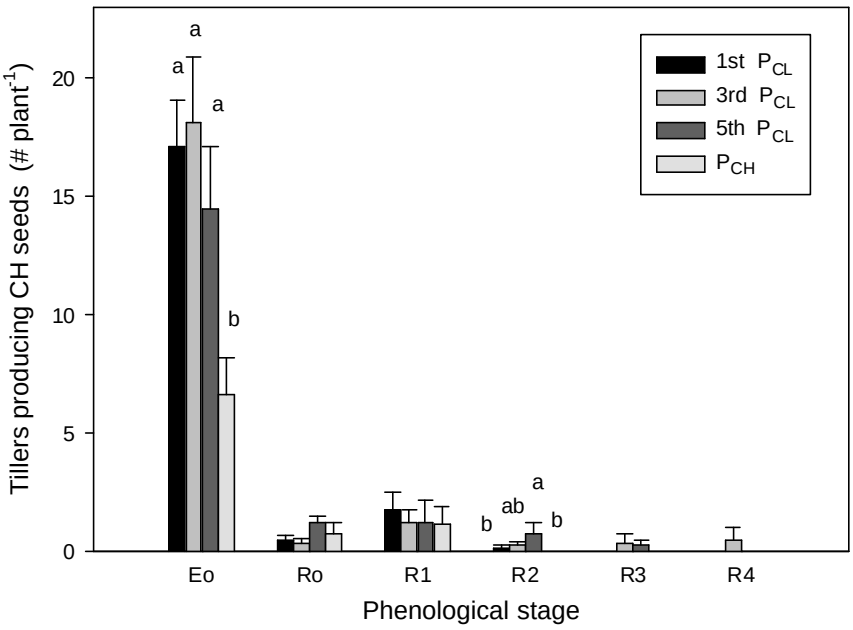

Figure 4.1 Phenological stages of tillers producing chasmogamous (CH) flowers in punagrass (*Achnatherum brachychaetum*) plants derived from cleistogamous seeds (P_{CL}) at different node positions (1st, 3rd and 5th) and chasmogamous seeds (P_{CH}). Measurements were taken 99 days after planting. Eo: onset of stem elongation; Ro: boot stage; R1: inflorescence emergence/first spikelet visible; R2: spikelets fully emerged/peduncle not emerged; R3: inflorescence emerged/peduncle fully elongated, and R4: anther emergence/anthesis. Values are means ± SE. Different lower case letters within each stage denote significant differences among progeny types (P = 0.05).

For flechilla grande, at 197 DAP, CH progeny had a significantly (P = 0.030) greater number of tillers producing CH seeds at the stage Ro (Booting) than CL progeny (Fig. 4.2). There were no differences in the number of tillers producing CH seeds between CH and CL progeny at stages Eo, So and S1.

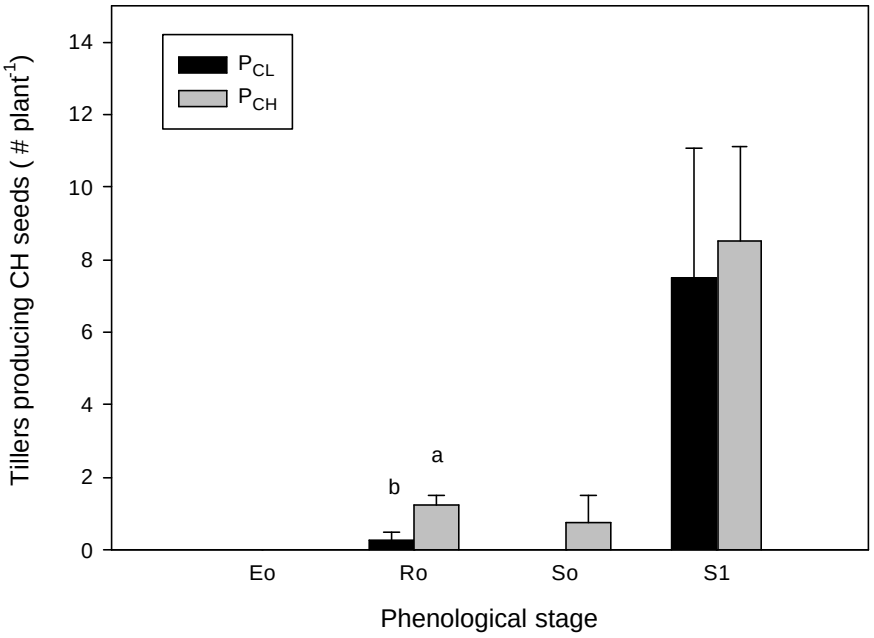

Figure 4.2 Phenological stages of tillers producing chasmogamous (CH) flowers in flechilla grande *(Nassella. clarazii*) plants derived from cleistogamous (P_{CL}) and chasmogamous (P_{CH}) seeds. Measurements were taken 197 days after planting. Eo: onset of stem elongation; Ro: boot stage; So: seed green and S1: seed ripe. Values are means ± SE. Different lower case letters within each stage denote significant differences (P = 0.05).

The morphological development measured by the Mean Stage Count (MSC) was similar among progeny types during early growth up to 99 DAP (Fig. 4.3). By 118 DAP, CH progeny matured earlier than CL progeny and was more than 3 weeks ahead of CL 1st and 5th node progeny.

The main effect of time of measurement was highly significant (P < 0.001), and the progeny effect on MSC was significant (p = 0.019) in the linear contrast. At the end of the experiment, a slightly significant effect due to progeny type was found (P = 0.08), as CH progeny achieved the highest MSC.

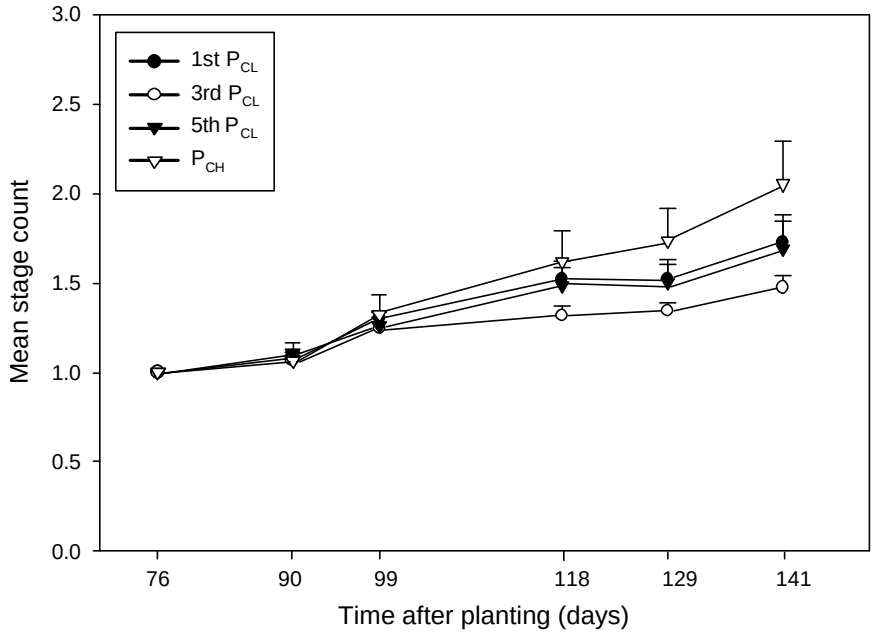

Figure 4.3 Adapted mean stage count (MSC) of punagrass (*Achnatherum brachychaetum*) plants derived from cleistogamous seeds (P_{CL}) at different node positions (1^{st}, 3^{rd} and 5^{th}) and chasmogamous seeds (P_{CH}). The following growth stages were used for constructing the index: vegetative stage, onset of stem elongation, boot stage, inflorescence emergence/first spikelet visible, spikelet fully emerged/peduncle not emerged, inflorescence emerged/peduncle fully elongated, anther emergence/anthesis, developing seed/caryopsis visible, and seed ripe. Values are means ± SE.

The percentage of tillers per plant with CH seeds was affected by progeny type in punagrass (P = 0.028) (Table 4.1). Total tillers per plant were similar among progeny types, even though CH derived plants tended to produce fewer tillers than any other progeny (Appendix Table A1). Relative growth rate of the initial growth period to 76 DAP was affected by progeny type (P = 0.002). Height of CH progeny developed approximately 67% faster than that of CL progeny (Table 4.1). Moreover, final plant height was also affected by progeny type (P = 0.032), and CL progeny from the 5^{th} node produced taller plants than CH progeny.

Table 4.1 Attributes of punagrass (Achnatherum *brachychaetum*) plants derived from cleistogamous seeds (P_{CL}) at different node positions (1st, 3rd and 5th) and chasmogamous seeds (P_{CH}). DAP: days after planting. Means with the same letters within a row (attribute) are not significantly different at P =0.05.

Attribute	P_{CL} 1st	P_{CL} 3rd	P_{CL} 5th	P_{CH}
Plant height at 118 DAP (cm)	116 ab	100 b	128 a	100 b
Relative Growth Rate of height at 76 DAP (vegetative stage) (cm cm^{-1} d^{-1})	0.029 b	0.025 b	0.028 b	0.045 a
Tillers with CH seeds per plant at harvest (%)	30 ab	21 b	29 ab	44 a

For most of the attributes of flechilla grande, there were no significant differences between plants derived from CH and CL seeds (Appendix Table A2) except for plant height at 118 DAP (P = 0.039). CL progeny were taller than CH progeny, being 74 and 67 cm, respectively.

4.3.2 Phenology, developmental morphology and growth of CH progeny from areas with contrasting range condition

The only significant difference in vegetative attributes between range condition was plant height at 69 DAP, where plants from GC area were 9 cm taller (P = 0.013) than those from PC area (Table 4.2). Plants from GC area had 46% more tillers with CH seeds per plant than those from PC area (P = 0.022). This difference was not reflected in the percentage of tillers with CH seeds per plant (Appendix Table A6), which may be partially due to the fact that plants from both areas did not differ in total number of tillers per plant (Appendix Table A3).

Table 4.2 Attributes of chasmousgamously (CH) derived plants of punagrass (*Achnatherum brachychaetum*) from seeds collected in areas of contrasting range conditions (Poor and Good). DAP: days after planting. Means with the same letters within a row (attribute) are not significantly different at P =0.05.

Attribute	Range condition	
	Poor	Good
Plant height at 69 DAP (cm)	66 b	75 a
Tiller with CH seeds per plant at harvest	13 b	19 a

4.3.3 Seed production of CH and CL progeny from different node positions

By comparing the seed production of mature tillers (producing CH seeds) vs. that of undeveloped young tillers, an early provision to CL seeds at the lowermost nodes of young tillers of punagrass was observed (Figs 4.4; 4.5). Differences among progeny types were not significant except for seed mass per node in the 2nd node of mature tillers, where CL progeny from the 3rd node had less seed mass than others ($P = 0.035$). Seed mass per node varied among nodes in the cubic model, with or without including the CH seeds of the terminal panicles ($P < 0.001$), and among progeny types for CL seeds ($P = 0.010$), also in the cubic model. Seed number per node was affected by node position in the cubic contrast, with or without including the CH seeds of the terminal panicles ($P < 0.001$), but it was not affected by progeny type. In young tillers, there was a decrease in total CL seed mass and number per node from the lower to the upper nodes along a tiller. In mature tillers, total CL seed mass per node decreased from the 2nd second node to the uppermost nodes. Total CL seed number per node increased from the 1st to the 3rd node, then decreased to the uppermost nodes. CH seed number and mass per tiller were greater than total CL seed number and mass per tiller only for the CL progeny from the 1st node ($P = 0.010$; $P = 0.002$) (Figs 4.4; 4.5).

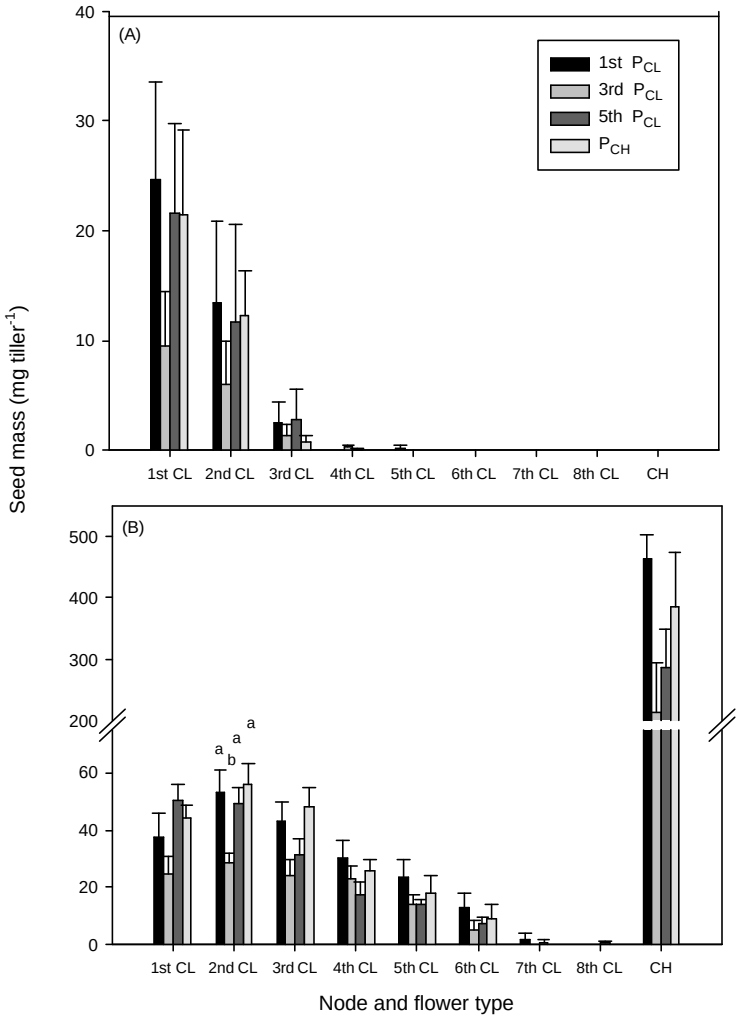

Figure 4.4 Effects of progeny type on cleistogamous (CL) seed mass at different nodes along the tiller and chasmogamous (CH) seed mass in (A) young, and (B) mature tillers (producing CH seeds) of punagrass (*Achnatherum brachychaetum*) plants derived from cleistogamous seeds (P_{CL}) at different node positions (1^{st}, 3^{rd} and 5^{th}) and chasmogamous seeds (P_{CH}). Values are means ± SE. Different lower case letters within each seed type denote significant differences (P = 0.05).

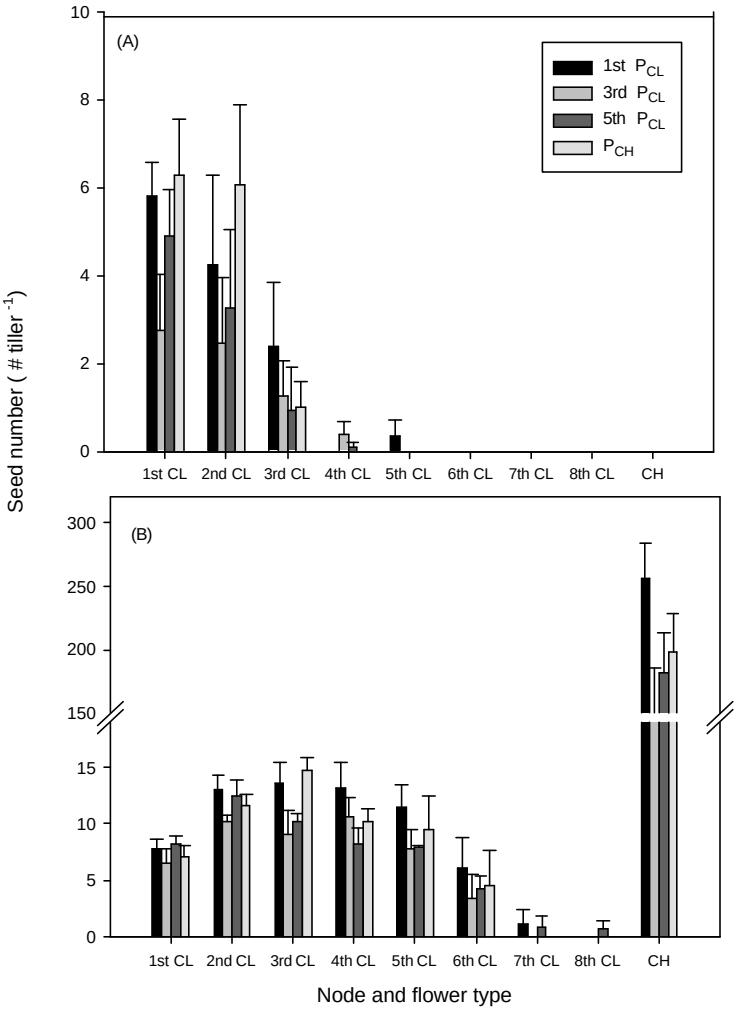

Figure 4.5 Effects of progeny type on cleistogamous (CL) seed number at different nodes along the tiller and chasmogamous (CH) seed number in (A) young, and (B) mature tillers (producing CH seeds) of punagrass (*Achnatherum brachychaetum*) plants derived from cleistogamous seeds (P_{CL}) at different node positions (1st, 3rd and 5th) and chasmogamous seeds (P_{CH}). Values are means ± SE. Different lower case letters within each seed type denote significant differences (P = 0.05).

Mass of individual seeds produced at the 1st and 2nd nodes were (P = 0.038; P = 0.042) affected by progeny, with CL 3rd node progeny having the smallest seed. Individual seed mass decreased from the lowermost node (1st CL) to the uppermost node (top CH) (Fig. 4.6), and it was affected by node position (P < 0.001) and progeny type (P = 0.024) in the linear contrast whether or not included CH seeds.

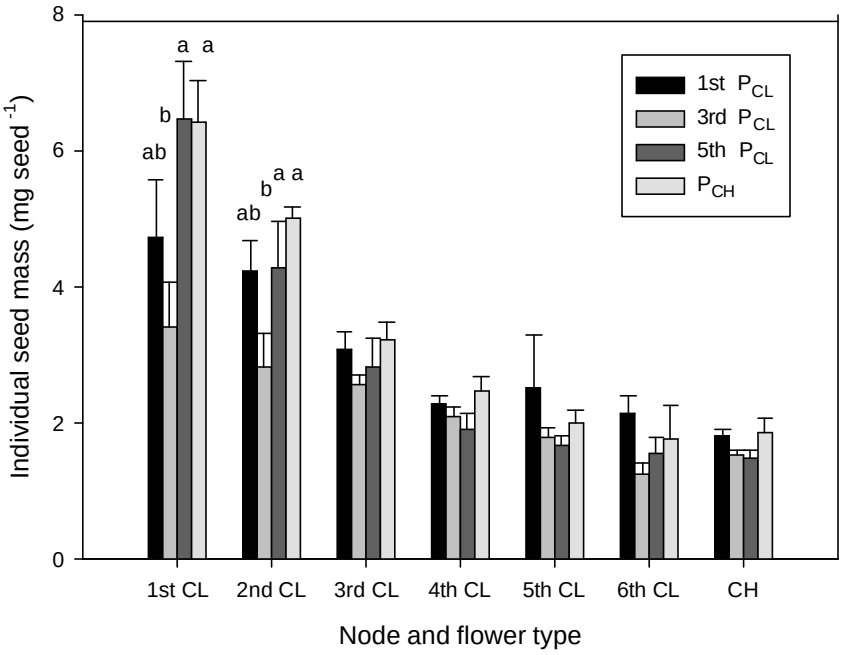

Figure 4.6 Effects of progeny type on cleistogamous (CL) individual seed mass at different nodes along the tiller and chasmogamous (CH) individual seed mass in punagrass (*Achnatherum brachychaetum*) plants derived from cleistogamous seeds (P_{CL}) at different node positions (1^{st}, 3^{rd} and 5^{th}), and chasmogamous seeds (P_{CH}). Values are means ± SE. Different lower case letters within seed type denote significant differences (P = 0.05).

Seed production per plant did not vary between CL and CH progeny types, possibly due to the large variation among replications, CH seed mass per plant tended to be higher for CL 1^{st} node progeny than for others (P = 0.09) (Appendix Table A4). In particular, CH seed mass per plant of CH progeny had a larger variation among replications than that of CL progeny.

In flechilla grande, CH progeny produced (P = 0.019) larger seeds than CL progeny (Fig. 4.7). Moreover, CH seeds were (P = 0.018) bigger than CL seeds. CL seed production was low (Appendix Table A5), as plants did not produce CL seeds in most of the tillers. Total seed number and mass, CH seed number and mass and CL seed number and mass per tiller and per plant were similar in CH and CL progeny (Appendix Table A5).

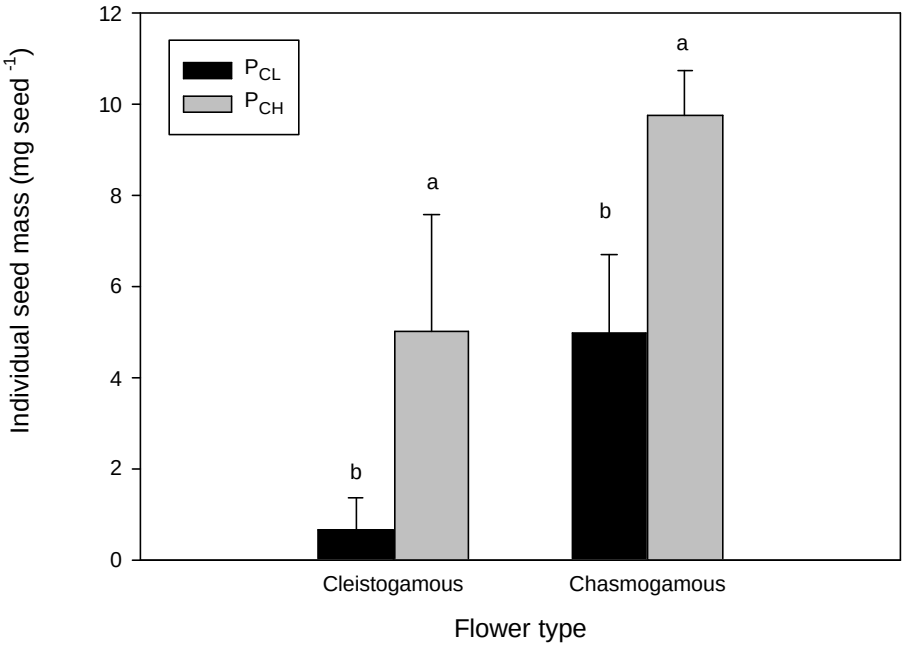

Figure 4.7 Effects of progeny type on cleistogamous (CL) and chasmogamous (CH) individual seed mass in flechilla grande *(Nassella clarazii*) plants derived from cleistogamous (P_{CL}) and chasmogamous (P_{CH}) seeds. Values are means ± SE. Different lower case letters within each flower type node denote significant differences (P = 0.05).

4.3.4 Seed production of CH progeny from areas with contrasting range condition

CL seed mass per tiller at the 2nd node was affected (P = 0.032) by range condition (Fig. 4.8). CL seed mass per tiller at the second node was higher in the GC area. CL seed mass across nodes was significantly (P = 0.02) affected by range condition in the cubic model. Seed number per tiller to either CH or CL seeds at any node and over nodes in punagrass were not affected by range condition (Fig. 4.9).

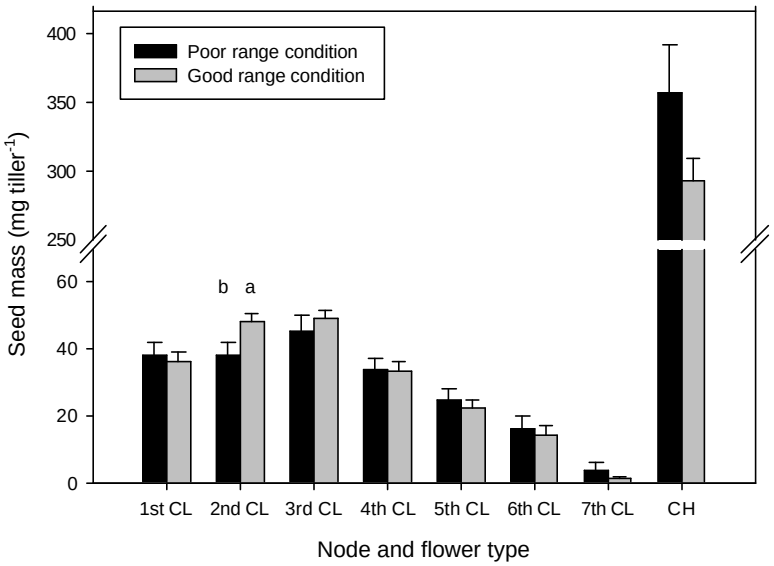

Figure 4.8 Effects of range condition on cleistogamous (CL) seed mass at different nodes along the tiller and chasmogamous (CH) seed mass in punagrass (*Achnatherum brachychaetum*) plants derived from chasmogamous seeds collected in areas of contrasting range conditions (Poor and Good). Values are means ± SE. Different lower case letters within seed type denote significant differences among different range conditions (P = 0.05).

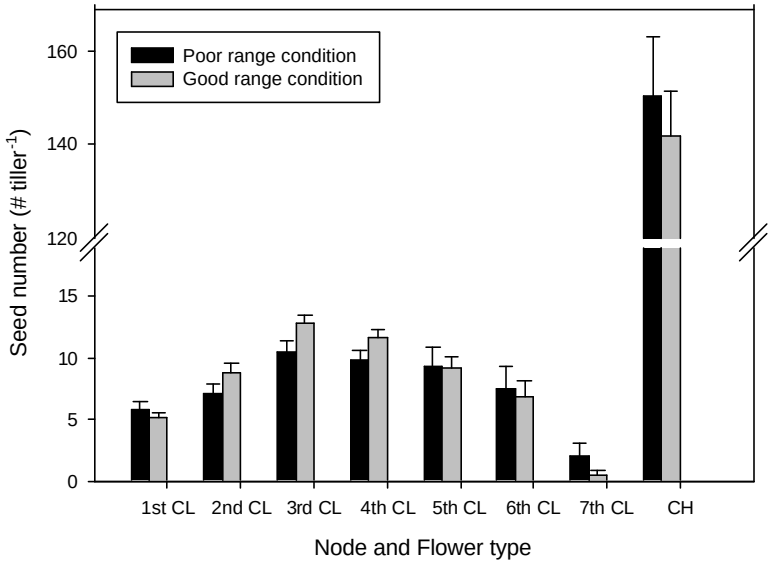

Figure 4.9 Effects of range condition on cleistogamous (CL) seed number at different nodes along the tiller and chasmogamous (CH) seed number in punagrass (Achnatherum brachychaetum) plants derived from chasmogamous seeds collected in areas of contrasting range conditions (Poor and Good). Values are means ± SE. Different lower case letters within seed type denote significant differences among different range conditions (P = 0.05).

Plants from PC areas had heavier (P = 0.031) CH seeds than those from GC areas (Fig. 4.10). Across all nodes, individual seed mass was affected (P < 0.05) by range

condition whether or not CH seeds were included (P = 0.041; P = 0.019), as indicated by the quadratic models.

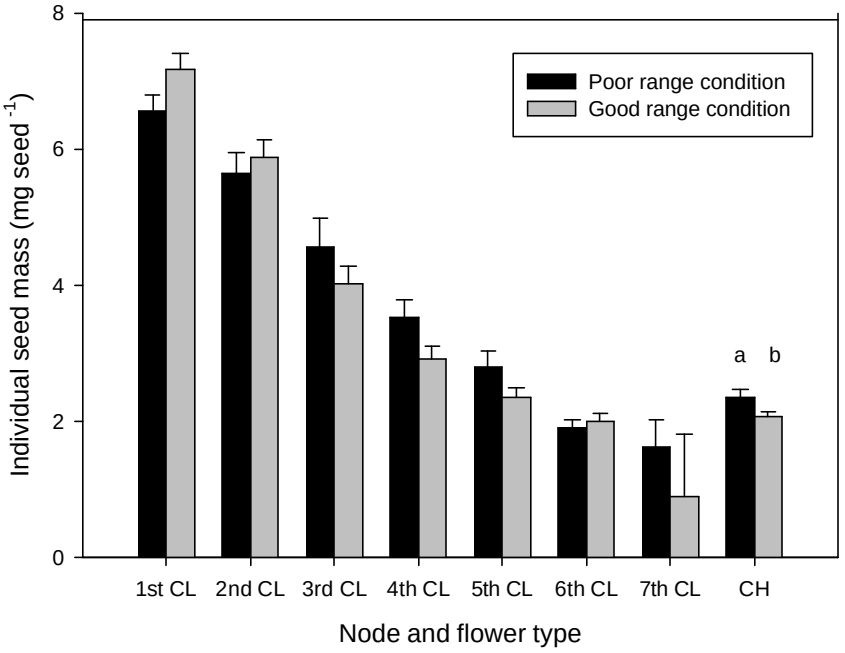

Figure 4.10 Effects of range condition on cleistogamous (CL) individual seed mass at different nodes along the tiller and chasmogamous (CH) individual seed mass in punagrass (*Achnatherum brachychaetum*) plants derived from CH seeds collected in areas of contrasting range conditions (Poor and Good). Values are means ± SE. Different lower case letters within each seed type denote significant differences among different range conditions (P = 0.05).

Total CL seed production per plant was greater in the GC than the PC area both in terms of total CL seed number (P = 0.002) and CL seed mass (P = 0.001) per plant (Table 4.3). Progeny from GC area also tended to have more seeds per plant (P = 0.073) and total seed mass per plant (P = 0.089) (Appendix Table A6). Total and CH seed production per plant were highly variable, especially for plants from GC, while the variability of CL seed production was low in both areas. Total seed number per plant was between 1,692 and 5,575 in PC, and 1,950 and 8,221 in GC. Total seed mass per plant varied from 5.8 g to 15.5 g in PC and from 5.7g to 22.9 g in GC. CH seed number per plant ranged from 807 to 3,204 seeds in PC, and from 1014 to 5250 seeds in GC, while that CH seed mass per plant varied from 2.1 g to 9.4 g in PC, and from 2.3 g to 10.7 g in GC.

Table 4.3 Effects of range condition on attributes of chasmousgamously derived plants of punagrass (*Achnatherum brachychaetum*) from seeds collected in areas of contrasting range conditions (Poor and Good). CH: chasmogamous. CL: cleistogamous. Means with the same letter within a row (attribute) are not significantly different at P =0.05.

Attribute	Range condition	
	Poor	Good
CL seeds per plant	1,215 b	1,619 a
CL seed mass per plant (mg)	4,425 b	5,971 a

4.3.5 Reproductive allocation to CH and CL seeds in CH and CL progeny from different node positions

Progeny type did not affect reproductive allocation to either CH or CL seeds at any node and across nodes in punagrass (Fig. 4.11). Reproductive allocation to CH seeds was significantly (P = 0.002) greater than to total CL seeds for CL 1st node progeny, as plants allocated 63% more biomass to CH (Mean 11.1 ± SE 0.95) than to CL seeds (Mean 6.8 ± SE 1.37).

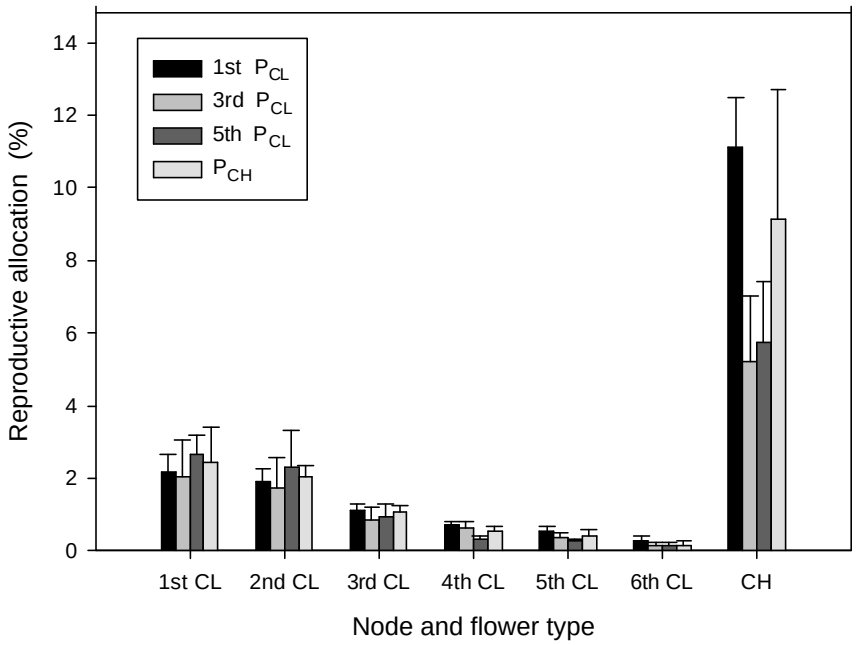

Figure 4.11 Effects of progeny type on reproductive allocation to cleistogamous seeds (CL) at different nodes along the tiller and chasmogamous seeds (CH) in each node in punagrass (*Achnatherum brachychaetum*) plants derived from cleistogamous (P_{CL}) at different node positions (1st, 3rd and 5th) and chasmogamous (P_{CH}) seeds. Values are means ± SE. Different lower case letters within each seed type denote significant differences (P = 0.05).

For flechilla grande, reproductive effort was very low and reproductive allocation to either CH or CL seeds was not affected by progeny (Appendix Table A5).

4.3.6 Reproductive allocation to CH and CL seeds in CH progeny from areas with contrasting range condition

Range condition did not affect reproductive allocation to CH or CL seeds of CH derived plants of punagrass (Fig. 4.12). Nevertheless, reproductive allocation to CL seeds was greater (P = 0.005) than to CH seeds. Plants from GC allocated more biomass to CL than to CH seeds (P < 0.001). Reproductive allocation was similar for CH or CL seeds for the plants from PC areas, in agreement with previous results (4.3.5).

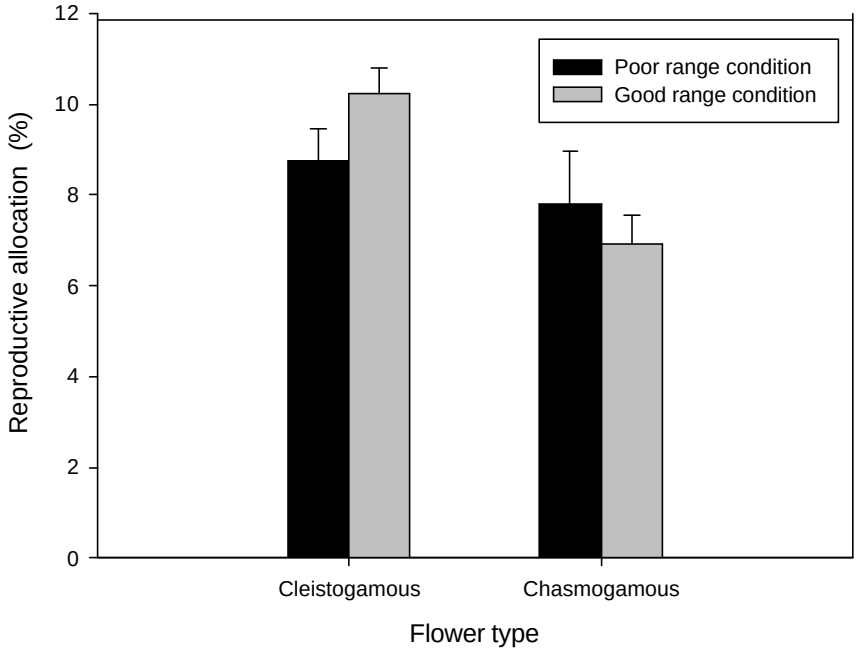

Figure 4.12 Effects of range condition on reproductive allocation to cleistogamous seed mass and chasmogamous seed mass in punagrass (*Achnatherum brachychaetum*) plants derived from chasmogamous seeds collected in areas of contrasting range conditions (Poor and Good). Values are means ± SE. Different lower case letters within each seed type denote significant differences (P = 0.05).

4.3.7 Germination of seeds from CH and CL progeny

Very few seeds of Punagrass germinated two months after harvesting when tested at 20/10 °C, 25/15 °C, or 30/20 °C, 9-h/15-h. Seed germination of flechilla grande was not related to progeny type (Appendix Table A5). Germination was also low for

seeds of GC and PC areas, averaging 5% for the CL seeds from 5th node of GC, 0.25% for the CL seeds from 1st node of GC, and 0.25% for the CL seeds from 5th node of PC area.

4.4 Discussion

CH progeny of punagrass grew faster, developed quicker, and matured earlier than CL progeny, but produced fewer tillers and shorter plants than CL progeny. Plants from CH seeds grew lesser than plants derived from CL seeds in peanutgrass (Cheplick and Quinn 1982). Individuals that emerged from big lower node seeds were heavier than those from light, upper node seeds in *Catananche lutea* and *T. purpurea* (Ruiz de Clavijo, 1995; Cheplick and Wickstrom, 1999). In the perennial grass, *D. clandestine*, the CH progeny had greater total shoot biomass per plant than the CL progeny only in low-density mixture of CH and CL plants (Bell and Quinn, 1985). Therefore, outcrossed CH progeny of Punagrass behave more like plants in unpredictable but productive environments where disturbances occur, resulting in the rapid occupation of space, which is advantageous for future competition for limiting resources (Grime, 1979). Conversely, CL progeny showed characteristics of plants of low-resource and stressful environments, which consistently have a lower relative growth rate than plants from more favourable environments (Lambers *et al*, 1998). On the other hand, growth rates of CL progeny of punagrass and those of both progenies of Flechilla grande were comparable, suggesting that these plants may be adapted to unproductive habitats. The slow growth of CL progeny of punagrass can confer longer persistence of the populations in communities with limited resources, such as those at advanced stages of succession. Opportunistic responses of punagrass to new productive habitats can be due to its CH progeny. Previous reports show that punagrass is an invasive species common in overgrazed grasslands of Argentina (Cano, 1988, Llorens, 1995), degraded lands of Australia (Gardener and Sindel, 1998), and alfalfa pastures of Argentina and United States (Fuller, 1961; Ares *et al*, 1970b; Rodriguez, 1983; Canevari and Viss, 1998). Under conditions of selective and severe grazing, unpalatable species such as punagrass become more competitive (Moretto and Distel, 1999).

In flechilla grande, differences between CH and CL progeny in phenology, growth and developmental morphology were mostly not statistically different, except

that CH progeny produced more tillers at the booting stage and taller plants than the CL progeny. Therefore, contribution of the CH and CL progeny to growth and development of flechilla grande cannot be differentiated at adult stages. Probably, fitness differences between CH and CL progeny in flechilla grande arise from comparisons at seedling stages.

Distinct seed types maturing at different positions on the maternal plant in species with heteromorphism often differ in seed number and mass (Venable, 1985; Cheplick and Clay, 1989; Kigel 1995; Gutterman, 2000). Punagrass exhibited seed heteromorphism in individual seed mass which decreased from the lower to the upper nodes along tillers.

Seed heteromorphism in punagrass was also expressed in total CL seed mass and number per node along the tiller, particularly between CH and CL seed production. Similar patterns have been observed in other perennial grasses (Cheplick and Clay, 1989) and one annual grass (Cheplick, 1996b). Proximate factors related to physiological and developmental mechanisms and ultimate factors related to ecological interactions can cause seed heteromorphism in plants. Position effects on seed mass and number often results from temporal variation in reproductive development (Silvertown, 1984; Cheplick and Clay, 1989; Gutterman, 2000). Moreover, the observed variation in seed mass within a species has been attributed to the limited capacity of the machinery of seed provisioning (Vaughton and Ramsey, 1998; Leisman *et al*, 2000), and to variability of competition seedling are exposed to or frequency –dependent effects (Leisman *et al*, 2000).

Progeny type influenced total CL seed mass per node and individual seed mass in punagrass. Progeny of the 3rd CL seeds produced seeds with smaller individual mass than other progeny at the 1st and 2nd nodes. On the other hand, CL progeny of flechilla grande produced smaller CH and CL seeds than CH progeny. Within-species studies indicate that seedling size often increases with seed mass, and such difference in seedling size can affect fitness via the subsequent growth and survival of individuals (Stanton, 1984; Wulff, 1986; Vaughton and Ramsey, 1998).

High fitness was observed in plants of CL progeny from the 1st node of punagrass, as they had more CH seeds and greater CH seed mass per tiller respect to CL

seed production than other progenies. A clear fitness advantage has been shown in species where CL seeds are larger than CH seeds. Plants derived from small CH seeds produced fewer seeds than those from large CL seeds in peanutgrass (Cheplick and Quinn, 1982). Individuals that emerged from big CL seeds produced more seeds than those from light CH seeds in *T. purpurea* (Cheplick and Wickstrom, 1999). Under optimum growth conditions, the large-seeded plants of the CL progeny of Punagrass were able to produce many seeds with high potential for colonization.

Reproductive allocation to CH seeds was significantly greater than to CL seeds for CL 1st node progeny in Punagrass. Therefore, CL progeny allocated more resources to increase production of CH seeds. Greater resource allocation to reproduction was noted in the selfed progeny in peanutgrass (Cheplick and Quinn, 1982; Cheplick and Quinn, 1986). Thus, CL progeny of punagrass had higher relative vigour than CH progeny not only in CH seed production but also in total investment in CH seeds.

Total CH seed mass per plant of CH progeny had more variation among replications than that in CL progeny. Previous studies indicate that CH reproduction is more plastic than CL seed reproduction and affected more by environmental conditions (Clay and Antonovics, 1985; Le Corff, 1993; Cheplick, 1994). Variation in CL seed production was low among replications in punagrass. The production of a consistent number of large seeds at the basal nodes ensures reproductive success in uncertain, stressful environments (Schoen and Lloyd, 1984).

Seed heteromorphism in flechilla grande contrasted with punagrass with CH seeds being larger than CL seeds in both progeny. The CH seeds of flechilla grande have a hygroscopic 6-8 cm long, awn (Cano, 1988) to aid seed burial (Garnier and Dajoz, 2001). On the contrary, CL seeds do not have special devices for dispersal and/or burial and have higher probability to locate close to the maternal plant. The CH seeds of flechilla grande were large and comparable to CL seeds of punagrass. Seed production of punagrass was greater than flechilla grande. Seed size determines the outcome of seedling competition with seedlings from large seeds being more competitive than seedlings of small seeds (Rees, 1997; Leishman *et al*, 2000; Leishman, 2001). As a climax species, flechilla grande is expected to produce few large seeds. On the other hand, this trait would be disadvantageous after disturbances such as heavy grazing,

which favour species such as punagrass with many small seeds and high potential for colonization.

CH progeny from GC grassland produced taller plants and more tillers with CH seeds than that PC grassland. However, more tillers producing CH seeds in GC did not translate into higher CH seed production compared to the PC grassland. On the other hand, punagrass from GC grassland produced more CL seeds than those from PC grassland, causing a shift in the mating system toward greater selfing. Previous reports show that mixed-mating systems can be modified by stress, toward greater selfing (Bell and Quinn, 1987; Le Corff, 1993; Cheplick, 1994; Bennington and McGraw, 1995; Culley, 2002; Steets and Ashman, 2004). If resources are available, additional seeds are matured on upper nodes including CH seeds (Cheplick, 1989; Cheplick, 1996b; Cheplick and Sung, 1998).

A large amount of genetic variation related to cleistogamy was found in one natural population of *Danthonia spicata*, indicating that the degree of cleistogamy has the potential to respond to natural selection (Clay, 1982). When individuals are subject to marked environmental fluctuations, or when progeny are subject to environments different from those of their parents, selection for adaptive plasticity in the breeding system may occur (Quinn, 1998). Relative levels of selfing and outcrossing can change with succession (Quinn, 1998). As succession progressed and resources became more limited, the ratio of CL to CH seeds markedly increased in *A. purshii* and *C. micans* (McNamara and Quinn, 1977; Le Corff 1993). Therefore, GC grasslands with limited resources enhance CL seed production in punagrass.

Punagrass from areas in good range condition allocated more of their resources to CL seeds than to CH seeds. In the perennial grass, *D. clandestinum*, the two most plastic populations whose plants had a relatively higher allocation to CL seed production at limited soil water were also the ones with the least soil water in the field (Bell and Quinn, 1987). In general, plants give priority to CL seed production under stressful conditions because CL flowers are cheaper to produce (Schemske, 1978; Waller, 1979), reducing energy investment for reproduction while favouring growth for competition (Bennington and McGraw, 1995).

4.5 Summary

CH and CL progeny in punagrass have different contributions to fitness, through specific mechanisms regulating reproduction. In contrast, CH and CL progeny of flechilla grande mostly have similar contributions to the plant fitness. Both species display reproductive strategies from contrasting environments. Punagrass exhibits a dual reproductive strategy with some characteristics of ruderal and others of competitive species whereas flechilla grande display clear traits of competitive species.

CH progeny of punagrass have the capacity for a fast development compared to CL progeny. CH progeny do not increase the production of CH seeds or allocate resources to produce more CH seeds in areas with low competitive pressure from palatable grasses. Nevertheless, CH progeny still produce many CH seeds. On the contrary, CH progeny show two mechanisms when facing competition: producing taller plants and reallocating resources in favour of CL seeds. CL seeds are larger, and have better chances for seedling survival, for producing more competitive seedlings, and persisting longer under adverse conditions than CH seeds. In fact, CL progeny grow and develop slower, with potential for persisting under limiting conditions. With high availability of resources (e.g., optimum conditions in the greenhouse), CL progeny produced more CH seeds, greater CH seed mass per tiller, and higher reproductive allocation per plant to CH than CL seeds. CL progeny of punagrass allows this species to persist in high seral plant communities and to produce many CH seeds when nutrients are available. Thus, large-seeded plants of punagrass from CL progeny have a fitness advantage for producing more flowers and seeds, which are perhaps more costly to produce. More diverse offspring in punagrass increases fitness of its populations and contribute to its high colonization capacity through different mechanisms: fast development of the CH progeny, and an opportunistic response of the CL progeny to produce the more dispersible CH seeds.

Flechilla grande produces few, large CH and CL seeds as compared to punagrass. These traits confer high competitive ability but these traits also are disadvantageous under heavy grazing, which favours species such as punagrass with high number of small seeds, high potential for colonization and large seeds for seedling competition. Although CL seeds of flechilla grande were smaller than CH seeds, they

did not show reductions in fitness of adult plants. CL seeds are located at the base of the plant, and protected from disturbances. A decrease in the number of CH flowers and seeds as a consequence of selective grazing on flechilla grande, can increase the relative importance of the CL seeds. Thus, the role of CL seeds in the regeneration of flechilla grande can be crucial in overgrazed grasslands of central Argentina.

5. EFFECTS OF MATERNAL NUTRIENT ENVIRONMENTS ON GROWTH AND REPRODUCTION OF PUNAGRASS AND FLECHILLA GRANDE

5.1 Introduction

For species with seed heteromorphism, the relative quantities of different seed types produced and their relative masses are influenced by environmental factors affecting the maternal plant (Weiss, 1980; Kawano *et al*, 1990; Cheplick, 1994; Kigel, 1995; Cheplick, 1996b; Cheplick and Sung, 1998; Gutterman, 2000). Maternal environments can also alter the balance between CH and CL seeds (Le Corff, 1993; Cheplick, 1994; Cheplick and Sung, 1998) and germination patterns (Weiss, 1980; Cheplick and Sung, 1998; Gutterman, 2000). For example, CH seed number and CL seed mass in annual grasses are enhanced by high soil fertility (Cheplick, 1989; Cheplick, 1996b; Cheplick and Sung, 1998). Allocation to CL subterranean seeds was constant at two nutrient levels, while allocation to aerial CH seeds increased at high soil nutrient (Cheplick 1989). More resource allocation was devoted to CL than to CH seeds in grasses under limiting resources (McNamara and Quinn, 1977; Cheplick and Quinn, 1982).

A dual reproductive strategy with the production of CL seeds with poor dispersal potential and CH seeds with high dispersal potential depends on the availability of resources for maternal plants. In general, plants give priority to CL reproduction under stressful conditions because CL flowers are cheaper to make (Schemske, 1978; Waller 1979), and CH seed production increases when resources are highly available (Le Corff, 1993; Cheplick, 1994; Cheplick and Sung, 1998; Quinn, 1998). A reproductive strategy with limited dispersal in space, such as the production of big CL seeds can evolve when the cost of dispersal is high (Olivieri, 2002).

Grass species from nutrient-poor habitats tend to have a lower growth potential than species from nutrient-rich habitats when studied under optimal environmental conditions (Bradshaw *et al*, 1964; Lambers *et al*, 1998). Fast growth of ruderals allows them rapidly to occupy space, which pre-empts limiting resources and facilitates rapid completion of the life cycle of a plant (Grime, 1977). On the other hand, slow growth reduces the turnover rate of leaves and increases tolerance to nutrient low supply (Tilman and Wedin, 1991; Lambers *et al*, 1998). Plant invasion is dependent on the creation of bare ground, debilitation of preexisting vegetation (Thompson *et al*, 2001), and is correlated with the availability of unused resources (Davis *et al*, 2000). Invasibility can also be explained by seed size and germination characteristic of plants at early stages of invasion (Burke and Grime, 1996).

Punagrass is an unpalatable species adapted to lowlands of high fertility (Distel *et al*, 2003), common in overgrazed grasslands of Argentina (Cano, 1988; Llorens, 1995). The grass is invasive in pastures and croplands with fertile soils (Ares *et al*, 1970b; Caro and Sanchez, 1971; Garderner and Sindel, 1998). Flechilla grande is a dominant, climax species in Argentine grasslands and is expected to be competitive with nutrient shortage because it reduces biomass and seed production of nearby unpalatable grasses (Moretto and Distel, 1997). It is tolerant to defoliation and water stress (Flemmer *et al*, 2002), and has a higher nutrient uptake rate than unpalatable grasses (Saint-Pierre *et al*, 2004).

The purpose of this study was to compare the vegetative and reproductive response to increasing levels of nutrients of these two species, which both exhibit seed heteromorphism associated with cleistogamy and chasmogamy but differ in functional characteristics, competitive ability and palatability to herbivory. The objective was to determine the effect of increasing soil nutrients applied to maternal plants on growth, CH and CL seed production (seed mass and seed number), biomass production, reproductive allocation to CH and CL seeds and seed germinability of punagrass and flechilla grande. It was hypothesized that favourable nutrient environments 1) enhance growth and seed production more in punagrass than in flechilla grande; and 2) increase seed production and germinability of CH seeds in punagrass and flechilla grande.

5.2 Material and Methods

5.2.1 Seed collection

Seeds of punagrass and flechilla grande were collected from the dry caldén forest in the semiarid region of Province of La Pampa, Central Argentina. The area had been subject to heavy grazing and was in poor range condition with less than 25 % palatable grass cover. Please see Chapter 3 for more details of the study site.

Chasmogamous seeds of flechilla grande and punagrass were collected in December 2001 and 2002 (early summer), respectively. Seeds were obtained from maternal plants of similar size. Seeds of punagrass and flechilla grande were collected in four and three, respectively, randomly selected, homogeneous sites that had high densities of adult plants. For more details about seed collection, processing and storage, see Chapter 3.

5.2.2 Experimental design

Seedlings of punagrass and flechilla grande derived from CH seeds were used in this experiment. A Randomized Complete Block Design (RCDB) with eight replications and three nutrient treatments was used.

For germination tests, a Completely Randomized Design (CRD) with six seed types (CH seeds, and CL seeds from nodes 1^{st} to 5^{th}), three nutrient treatments, five replications and 25 seeds per experimental unit was used for punagrass. For CH seeds of flechilla grande, a CRD with three nutrient treatments, two seed treatments (hulled and dehulled), four replications and 25 seeds per experimental unit was used.

CH and CL seeds of flechilla grande and CH and CL from the 1^{st}, 2^{nd}, 3^{rd}, 4^{th} and 5^{th} nodes of punagrass were tested for viability. Three replications with 20 seeds per experimental unit and 15 seeds per experimental unit for the CL seeds of flechilla grande were used.

5.2.3 Plant growth conditions

Seedlings of 4-6 days old and of similar height were randomly selected from previous germination experiments (Chapter 3) and planted in plug flats (48 cells 9 cm^2 / flat TLC Polyform) containing a mixture of fine washed sand and topsoil (3:1). The

following soil properties were determined using 5 replicates: field capacity (%), pH (Beckman pH Meter), total organic carbon (%) (Combustion Method with LECO Carbon determinator CR-12), nitrates (Autoanalyzer Colorymeter, Pulse Instrumentation Ltd., 2:1 water extraction), potassium (Atomic Absorption Spectometer with Spectr AA 220 Varian), sulphates (Auto Analyzer Colorimeter, Technicom), and phosphorus (Kalowna Method) (Table 1).

Table 5.1 Physical and chemical properties of the soil mixture used in the experiments

Soil property	Determined value
Field Capacity (%)	17.8
pH	7.60
Total Organic Carbon (%)	0.26
NO_3 (ppm)	9.54
K (ppm)	17.1
SO_4 (ppm)	62.6
P (ppm)	0.70

Seedlings were watered daily to field capacity and left in the growth chamber for two weeks at 25/15 °C (9/15 h), 9-h light (400 Lux of white cool fluorescent)/15-h darkness. Trays with water were placed in the growth chamber to maintain moisture. Seedlings were then moved to a greenhouse, and transferred to pots (15 cm in diameter x 18 cm in depth) containing the same soil mixture. Seedlings were marked and randomly allocated to nutrient treatments.

Plants were subjected to one of three nutrient treatments, at 71 and 123 days after planting (DAP): 1) high-nutrient (simulating post-burning nutrient level): 60 ppm N and 90 ppm P; 2) moderate-nutrient (simulating pre-burning nutrient level): 43 ppm N and 63 ppm P; and 3) low nutrient: tap water. These nutrients were based on the results of a prescribed burning study conducted in the caldén forest of Argentina (Castelli and Lazzari, 2002).

Calcium nitrate [$Ca(NO_3)_2 . 4H_2O$] (Sigma Ltd.) and monocalcium phosphate [$Ca(H_2PO_4)_2$] (J. T. Baker Ltd.) were used for the nutrient solutions. Each plant received 10 mL of nutrient solution per application. Plants were watered regularly to

field capacity with distilled water in the first 3 months and with tap water near the end of the experiment.

Since plants of flechilla grande did not flower under greenhouse conditions, they were placed in growth chambers after 180 DAP at 15/10 °C, light darkness (8/16 h) for two weeks, followed by 10/6 °C, light darkness (8/16 h) for another two weeks, and again at 15/10 °C light darkness (8/16 h) for one week. Plants were transferred back to the greenhouse after the treatments.

5.2.4 Data collection

The height of each plant, total leaf number, and tiller number per plant at the time of the 1st fertilization (71 DAP) were recorded for both species. These parameters were measured again at 126, 152 and 203 DAP. The following stages of reproductive phenology based on CH flowers and seeds were recorded for punagrass at 152, 170, 179, 190 and 203 (harvest time) DAP: stem elongation (3 nodes visible), boot swollen, first spikelet visible, spikelet fully emerged/peduncle not emerged, inflorescence emerged/peduncle fully elongated, anther emergence/anthesis, developing seed/caryopsis visible and seed ripe. The total number of tillers per plant at each stage and date was recorded. For flechilla grande, the number of tillers per plant presenting the following phenological stages was recorded at harvest time: stem elongation (3 nodes visible), boot stage, developing seed/caryopsis visible and seed ripe.

Since CH seeds mature early in punagrass, terminal panicles were harvested first. The remaining tillers were labelled and plants were given additional time for CL seeds to mature. Punagrass and flechilla grande plants were harvested after 203 and 300 DAP, respectively. Soil was washed off the roots and entire plants were placed into paper bags. Up to five tillers per plant that produced CH seeds in terminal panicles were sampled for both species. Once seeds were separated from the stems, plant materials were oven-dried at 85 °C to constant mass. The dry mass of each tiller was recorded separately. Roots were also oven-dried to constant mass under the same conditions.

Tillers of punagrass were separated by nodes and examined for the occurrence of CL seeds. Seed number and total fresh seed mass per tiller were recorded for each node, including the terminal panicles with CH seeds. For flechilla grande, seed number and total fresh mass of CH and CL seeds per tiller were recorded. CL seeds were classified

into fully developed, green and immature whereas that CH seeds into fully developed and immature. After hand cleaning, fresh mass of fully developed CH, immature CH and fully developed CL seeds was measured using a Microbalance (Cahn C-33). CH seeds of both species and CL seeds of flechilla grande were weighed with hulls (palea and lemma).

Seed germination was tested two months after harvesting. Both CH and CL seeds of punagrass, but only CH seeds of flechilla grande were tested due to a shortage of CL seeds in the latter. Seeds of punagrass were incubated at 20/10 °C in darkness (Eilberg and Soriano, 1972), and seeds of flechilla grande were incubated at 25/15 °C, 9/15 h in darkness (Cabeza *et al*, 1999). Seeds were pooled by treatments and placed in Petri dishes containing water-saturated filter paper. Petri dishes were put in closed containers to avoid moisture loss. The lemma and palea of CH seeds were removed in punagrass. In flechilla grande, seeds were tested with and without palea and lemma. Seeds were considered germinated when the radicle was at least 2 mm length. Seeds were checked weekly for germination for four weeks. A green safe light was used while checking germination.

The remaining seeds at the end of germination experiments and additional CL seeds of flechilla grande were tested for viability with a 0.1% solution of Tetrazolium salt (Grabe, 1970). CH and CL seeds were bisected longitudinally and half of the seeds were soaked in the solution and incubated at room temperature for 24 h. Seeds were considered viable when the whole embryo was stained. Evaluation was done under a magnifier glass.

5.2.5 Data Analysis

The Relative Growth Rate (RGR) of height at early growth stage was calculated as (ln [plant height after 126 DAP] - ln [initial plant height at the first fertilization])/ 126 DAP.

Only fully developed CH and fully developed plus green CL seeds were included in seed production analysis. Seed number and seed mass per node and tiller represent the average seed production per node (including the CH seeds of the terminal panicle) of the sampled tillers that produced CH seeds. When a node did not have seeds, seed number and mass were registered as zero and the average included zero values. The individual

seed mass of each node was calculated using the total seed mass divided by the number of seeds per node and tiller. The final individual seed mass was the average of tiller nodes with seeds; nodes that lacked seeds were not taken into account in the average. Total seed number, total CL seed number, total seed mass and total CL seed mass per tiller were calculated as the sum of the parameters in all the nodes of the tiller, and then averaged for all sampled tillers. Total CH seed number per plant was calculated as the average CH seeds per tiller * total number of mature (producing CH seeds) tillers. CL seed number per plant of the four lowest nodes was calculated as the average seed number per node * total number tillers. Seed number of the rest of the nodes was calculated as the average seed number per node * total number of mature tillers. CH and CL seed mass per node per plant was calculated in the same way as seed number.

Reproductive allocation per node per plant was calculated as the percentage of seed mass per node per plant in total plant biomass, which included total seed mass per plant + total tiller biomass+ root biomass. CH reproductive allocation per plant was calculated as the percentage of CH seed biomass per plant in the total plant biomass.

Parameters for vegetative development, reproductive phenology, seed production and reproductive allocation were analysed using GLM of SAS system (SAS Institute Inc., Release 8.02). GLM with Type III sum of squares were used to analyze the effect of nutrient (fixed effect) within each phenological stage, date, flower type, or node position. Block term was treated as a random effect. Prior to each analysis, data were examined for normality and homocedasticity. The percentage of reproductive tillers per plant and reproductive allocation were arcsine square –root transformed to conform model assumptions (Underwood, 1997). Specific single degree of freedom orthogonal contrasts were used to compare: a) control vs. moderate nutrient treatment; b) control vs. high nutrient treatment; and, c) moderate vs. high nutrient treatment. Thus, variance (treatment sum of squares) due to increasing levels of a gradient factor (nutrient) was partitioned into meaningful components (Little, 1981). In addition, Fisher' least significant difference (LSD) test was performed with significance level at $P = 0.05$ (Steel and Torrie, 1997). For flechilla grande, the model included nutrient and flower type as fixed effects and block as a random effect.

For punagrass, seed number, reproductive allocation, and individual seed mass were further analysed using node position as repeated measures on the experimental unit or subject (plant). These variables may be correlated across observations (positions) on the same subject (Schabenberger and Pierce, 2002). The Durbin-Watson statistic was used as a measure of serial correlation (SAS Institute Inc., Release 8.02). Positive autocorrelation across node positions in seed number, individual seed mass and reproductive allocation was highly significant ($P < 0.001$) when CH plus CL seeds of all nodes were included. Correlations were also significant ($P < 0.001$) for seed number per node, individual seed mass and reproductive allocation when only nodes with CL seeds were included. Therefore, measurements of the variables across seed positions were considered repetitions on the experimental unit with correlated residuals. Repeated measures analysis was performed using the GLM procedure with node position as within-subject effect and nutrient treatment as between-subject effect. Reproductive allocation data were arcsine square –root transformed to meet model assumptions. The orthogonal polynomial transformation option was selected to implement the analysis. This transformation allows studying the nature of a repeated measures effect and accounts for substantial portions of the error variance over the repeated measurements, and correlations among residuals can be heterogeneous (Rowell and Walters, 1976; Gurevitch and Chester, 1986). GLM was used to test the n^{th} degree contrasts. The Type I F-test or sequential sum of squares was used for performing backward selection process using only a model fit; higher order terms were considered first and then dropped them from the model if they were not significant. Because the previous analysis did not show a significant block effect, the block factor was not included in the Model statement.

Final germination percentage was tested with a factorial analysis of variance using the GLM procedure in SAS. To meet model assumptions, data were arcsine square –root transformed (Underwood, 1997). Fisher' least significant difference (LSD) test was performed with a significance level at $P = 0.05$. For punagrass, an additional analysis was performed considering combinations of seed position and treatment factors with a total of 18 individual treatments (six seed positions x three maternal nutrient treatments). Specific orthogonal linear contrasts of interest were used for the last analysis.

5.3 Results

5.3.1 Effects of maternal nutrient environments on growth, seed production, biomass production and reproductive allocation to CH and CL seeds in punagrass and flechilla grande

For punagrass, nutrients had a significant ($P < 0.001$) effect on height, tiller and leaf number, and tiller and root biomass (Table 5.2). Increasing nutrient availability from moderate to high did not affect height, tiller and leaf number, but enhanced tiller and root biomass ($P < 0.05$). Root biomass was increased by high nutrient ($P < 0.001$), but not by moderate nutrient levels.

Table 5.2 Effects of nutrient treatments on vegetative attributes in plants of punagrass (*Achnatherum brachychaetum*) derived from chasmogamous seeds. C: control, M: moderate nutrients, and H: high nutrients. DAP: days after planting. Data presented are the probabilities of significance of orthogonal linear contrasts. NS = not significant.

Variable	C	M	H	Control vs. Moderate	Control vs. High	Moderate vs. High
Height at 203 DAP (cm)	38	60	71	< 0.001	< 0.001	NS
Tiller per plant at 203 DAP	46	92	125	0.001	< 0.001	NS
Leaves per plant at 203 DAP	106	210	295	< 0.001	< 0.001	NS
Average tiller biomass (g)	0.18	0.56	0.79	< 0.001	< 0.001	0.003
Average root biomass (g)	1.1	2.3	4	NS	< 0.001	0.015

For flechilla grande, high and moderate nutrient treatments enhanced height, leaf and tiller number per plant ($P < 0.05$). Tiller and root biomass were not influenced by nutrients (Table 5.3).

RGR of plant height of punagrass and flechilla grande responded differently to nutrient treatments (Fig. 5.1). The RGR of height of punagrass was approximately twice as great in the moderate and high nutrient treatments as in the control ($P < 0.001$), but there was no significant difference between moderate and high nutrient treatments. The RGR of height of flechilla grande was not affected by nutrient treatment.

Table 5.3 Effects of nutrient treatments on vegetative attributes in plants of flechilla grande (*Nassella clarazii*) derived from chasmogamous seeds. C: control, M: moderate nutrients, and H: high nutrients. DAP: days after planting. Data presented are the probabilities of significance of orthogonal linear contrasts. NS = not significant.

Variable	C	M	H	Control vs. Moderate	Control vs. High	Moderate vs. High
Height (cm) at 203 DAP	44	59	59	0.020	0.004	NS
Tillers per plant at 203 DAP	21	25	59	0.025	0.006	NS
Leaves per plant at 203 DAP	42	99	136	0.010	0.005	NS
Average tiller biomass at 300 DAP (g)	0.9	1.4	1.3	NS	NS	NS
Average root biomass at 300 DAP (g)	1.1	1.7	2.5	NS	NS	NS

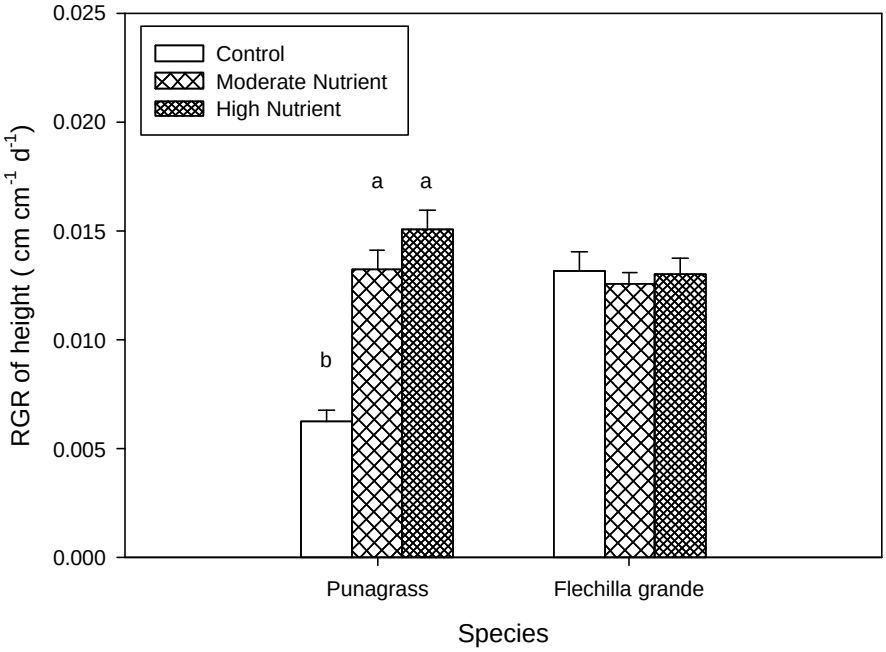

Figure 5.1 Effects of nutrient treatments on relative growth rate (RGR) in chasmousgamously derived plants of punagrass (*Achnatherum brachychaetum*) and flechilla grande (*Nassella clarazii*). RGR was calculated as (ln [height 126 Days after planting] - ln [initial height])/126 days. High nutrient (simulating post-burning nutrient concentration): 60 ppm N and 90 ppm P, Moderate nutrient (simulating pre-burning nutrient concentration): 43 ppm N and 63 ppm P, and Control: tap water. Values are Means ± SE. Different lower case letters denote significant differences among nutrient treatments within a species (P = 0.05).

For both species, similar effects of nutrients on tiller and leaf number development were observed after the second fertilization (123 DAP) (Figs 5.2, 5.3), but punagrass displayed a greater growth potential than flechilla grande in all nutrient

treatments. The effect of time on tiller and leaf number was significant ($P < 0.001$) for both species. Effect of nutrient treatment over time was also significant for both species ($P < 0.001$ and 0.05, respectively).

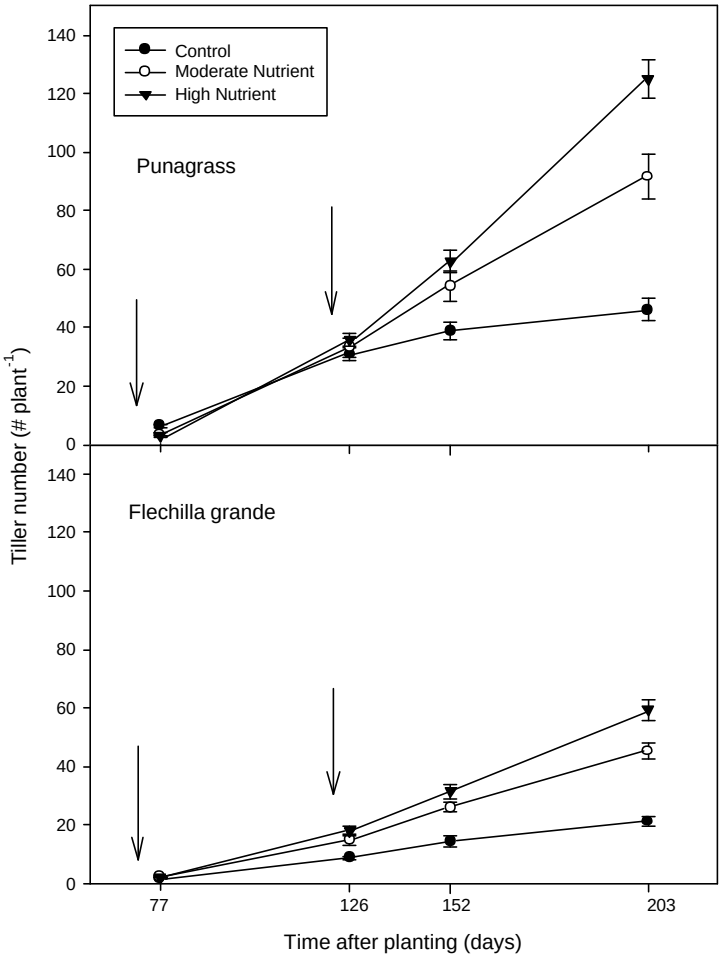

Figure 5.2 Effects of nutrient treatments on the number of tillers per plant in chasmousgamously derived plants of punagrass (*Achnatherum brachychaetum*) and flechilla grande (*Nassella clarazii*). High nutrient (simulating post-burning nutrient concentration): 60 ppm N and 90 ppm P, Moderate nutrient (simulating pre-burning nutrient concentration): 43 ppm N and 63 ppm P, and Control: tap water. Arrows indicate dates of fertilization. Values are Means ± SE.

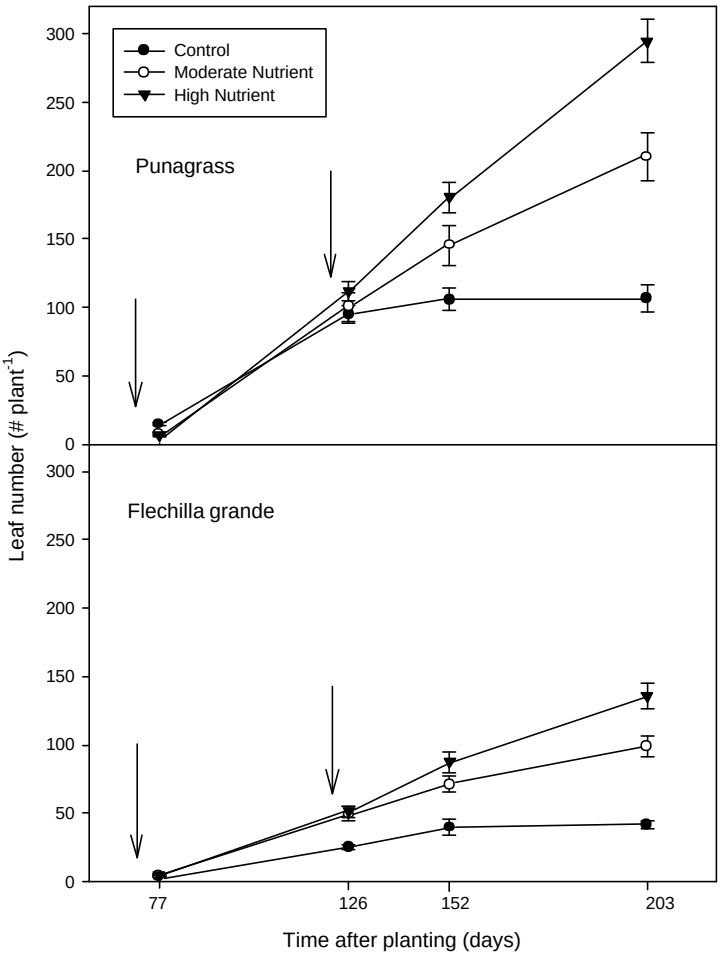

Figure 5.3 Effects of nutrient treatments on the number of leaves per plant in chasmousgamously derived plants of punagrass (*Achnatherum brachychaetum*) and flechilla grande (*Nassella clarazii*). High nutrient (simulating post-burning nutrient concentration): 60 ppm N and 90 ppm P, Moderate nutrient (simulating pre-burning nutrient concentration): 43 ppm N and 63 ppm P, and Control: tap water. Arrows indicate dates of fertilization. Values are Means ± SE.

Punagrass plants began to produce terminal panicles with CH seeds 152 DAP (Fig. 5.4). The number of tillers with CH seeds was lower in the control and moderate nutrient treatment than in the high nutrient treatment (Fig. 5.4). The effects of time and nutrient treatment on the number of tillers producing CH seeds were significant ($P < 0.001$). At the time of harvest (203 DAP), moderate and high nutrient treatments enhanced the number of tillers per plant with CH seeds (Table 5.4).

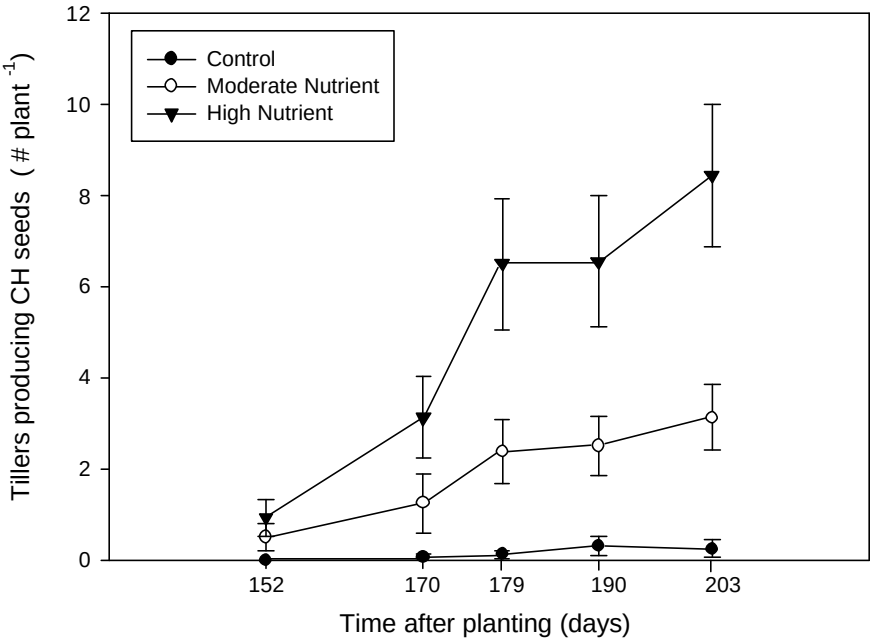

Figure 5.4 Effects of nutrient treatments on the number of reproductive tillers producing chasmogamous (CH) seeds in chasmousgamously derived plants of punagrass (*Achnatherum brachychaetum*). High nutrient (simulating post-burning nutrient concentration): 60 ppm N and 90 ppm P, Moderate nutrient (simulating pre-burning nutrient concentration): 43 ppm N and 63 ppm P, and Control: tap water. Values are Means ± SE.

At the time of harvest (203 DAP), the number of tillers producing CH seeds at all phenological stages were affected ($P < 0.05$) by nutrient treatments (Fig. 5.5). High nutrients promoted the production of tillers with CH seeds at all the stages and moderate nutrient levels at the onset of stem elongation.

For flechilla grande, high nutrient treatment increased ($P = 0.030$) the number of tillers producing CH seeds at the seed green and seed ripe stages (Fig. 5.5).

The percentage of punagrass tillers with CH seeds was greater ($P < 0.001$) in the high nutrient treatment compared to other treatments (Table 5.4).

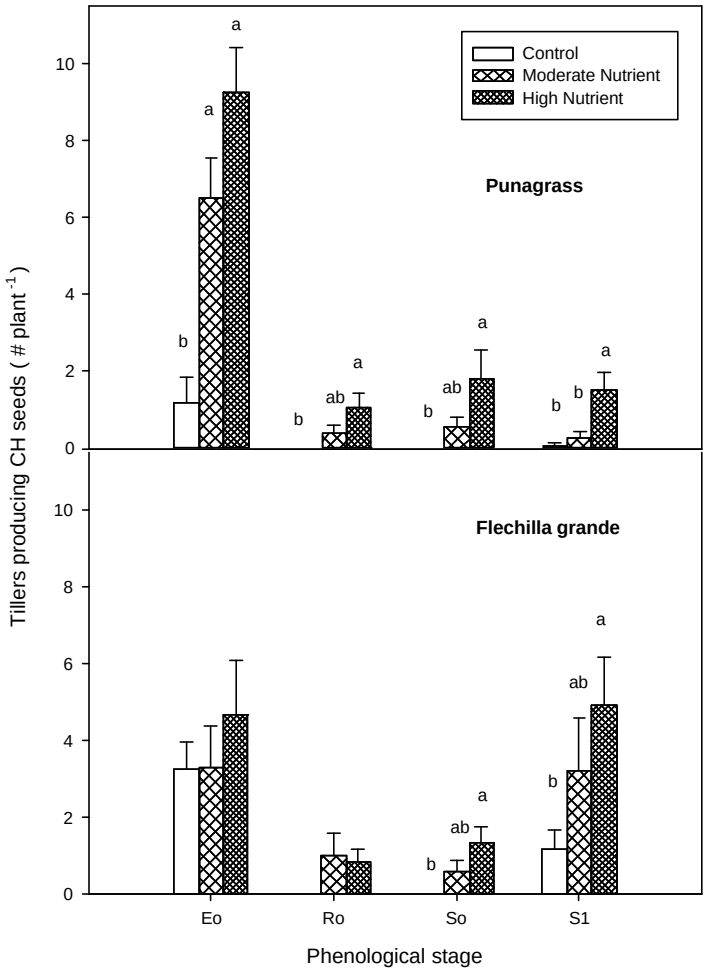

Figure 5.5 Effects of nutrient treatments on phenological stages of tillers producing chasmogamous (CH) seeds in chasmousgamously derived plants of punagrass (*Achnatherum brachychaetum*) and flechilla grande (*Nassella clarazii*) 203 and 300 days after planting, respectively. High nutrient (simulating post-burning nutrient concentration): 60 ppm N and 90 ppm P, Moderate nutrient (simulating pre-burning nutrient concentration): 43 ppm N and 63 ppm P, and Control: tap water. Eo: onset of stem elongation; Ro: boot stage; So: seed green and S1: seed ripe. Different lower case letters denote significant differences among nutrient treatments within a species (P = 0.05).

Total seed number and mass per tiller, total CL seed number and mass per tiller and total CH seed number and mass per tiller were increased (P<0.05) by moderate and high nutrient in punagrass (Table 5.4, Fig. 5.6).

For flechilla grande, the high nutrient treatment increased the number of tillers with CH seeds per plant (P = 0.020) while it did not significantly affect CL seed mass per tiller, total seed number per tiller, and percentage of tillers with CH seeds per plant (Table 5.5). Total seed mass and total CH seed mass per tiller were higher in the control treatment as compared to other treatments (P < 0.05) (Table 5.5). Total seed mass per

tiller and CH seed mass per tiller also were lower in the moderate nutrient treatment than the control. Previous response (Table 5.3) indicate that the moderate and high nutrient treatments increased number of tillers per plant, therefore, flechilla grande responded to increased nutrient by producing more shoots, but the percentage of these shoots that were reproductive remained constant.

Table 5.4 Effects of nutrient treatments on reproductive attributes in plants of punagrass (*Achnatherum brachychaetum*) derived from chasmogamous seeds. C: control, M: moderate nutrients, and H: high nutrients. Data presented are the probabilities of significance of orthogonal linear contrasts. NS = not significant.

Variable	C	M	H	Control vs. Moderate	Control vs. High	Moderate vs. High
Tillers with CH seeds per plant at harvest	0	4	10	NS	< 0.001	0.005
Percentage of tillers per plant with CH seeds at harvest	1	4	9	NS	< 0.001	0.023
Seed number per tiller	7	52	99	0.017	< 0.001	0.013
Number of cleistogamous seeds per tiller	6	30	49	0.003	< 0.001	0.013
Seed mass per tiller (mg)	38	157	277	0.019	< 0.001	0.019
Chasmogamous seed mass per tiller (mg)	6	57	97	0.042	0.001	0.010
Cleistogamous seed mass per tiller (mg)	32	100	180	0.036	< 0.001	0.017

Table 5.5 Effects of nutrient treatments on reproductive attributes in plants of flechilla grande (*Nassella clarazii*) derived from chasmogamous seeds. C: control, M: moderate nutrients, and H: high nutrients. Data presented are the probabilities of significance of orthogonal linear contrasts. NS = not significant.

Variable	C	M	H	Control vs. Moderate	Control vs. High	Moderate vs. High
Tillers with CH seeds per plant at harvest	5	8	12	NS	0.020	NS
Percentage of tillers per plant with CH seeds at harvest	24	18	20	NS	NS	NS
Seed number per tiller	5	4	5	NS	NS	NS
Seed mass per tiller (mg)	64.1	21.2	31.0	0.002	0.001	NS
Chasmogamous seed mass per tiller (mg)	56.6	15.0	24.2	0.002	0.001	NS
Cleistogamous seed mass per tiller (mg)	7.4	6.2	6.8	NS	NS	NS

For punagrass, seed number and individual seeds mass across nodes were affected by increasing availability of nutrients ($P < 0.001$) (Figs 5.6, 5.7). The nutrient

effects on seed number and individual seed mass were different between CH seeds and CL seeds at the 1^{st}-2^{nd} nodes when analysed within each node and flower type (Figs 5.6 A, 5.7 A). CH seed number increased from moderate to high nutrients ($P < 0.001$), but the individual seed mass did not change. On the contrary , the individual CL seed mass increased at the 1^{st}-2^{nd} nodes from moderate to high nutrients ($P < 0.001$), but seed number was unchanged. CL seeds from upper nodes (the 4^{th} to 6^{th} nodes) followed the same pattern as CH seeds.

Total CL seed number per tiller for flechilla grande decreased with increasing nutrients (Fig. 5.6 B). Total CH seeds per tiller remained constant, but the mass of CH seeds per tiller decreased with increasing nutrient levels (Table 5.5). Individual CH and CL seed mass were similar among nutrient treatments (Fig 5.7 B).

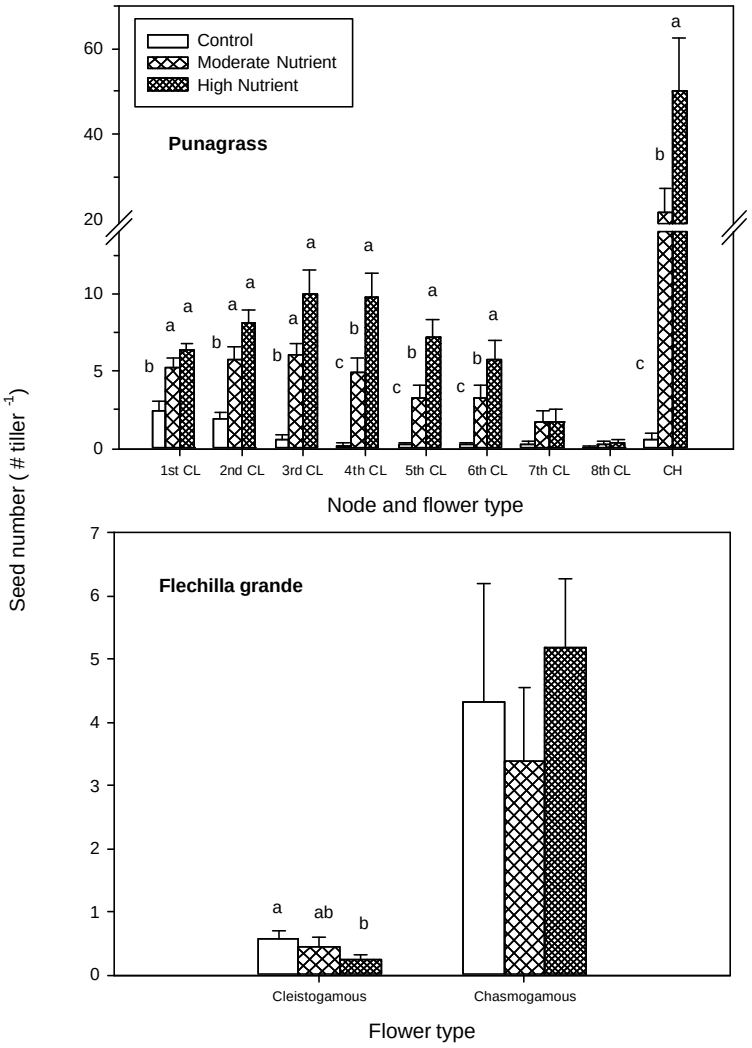

Figure 5.6 Effects of nutrient treatments on chasmogamous (CH) and cleistogamous (CL) seed number per tiller in each node in chasmousgamously derived plants of punagrass (*Achnatherum brachychaetum*) and flechilla grande (*Nassella clarazii*). High nutrient (simulating post-burning nutrient concentration): 60 ppm N and 90 ppm P, Moderate nutrient (simulating pre-burning nutrient concentration): 43 ppm N and 63 ppm P, and Control: tap water. Different lower case letters denote significant differences among nutrient treatments within a node position or flower type and a species (P = 0.05).

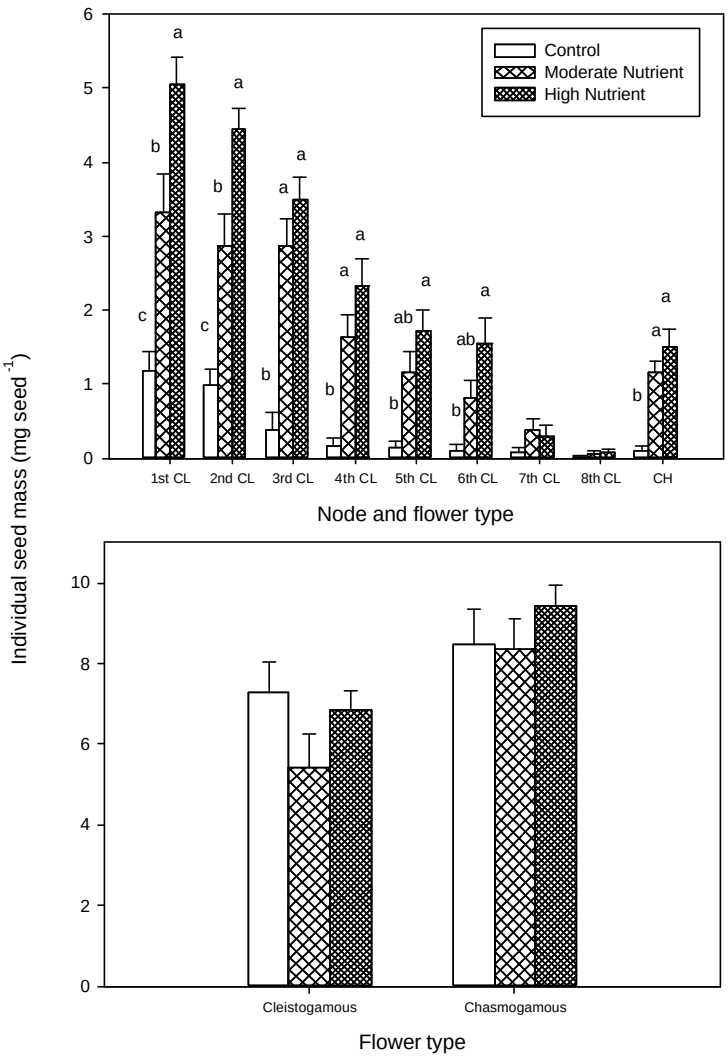

Figure 5.7 Effects of nutrient treatments on chasmogamous (CH) and cleistogamous (CL) individual seed mass of each node in chasmousgamously derived plants of punagrass (*Achnatherum brachychaetum*) and flechilla grande (*Nassella clarazii*). High nutrient (simulating post-burning nutrient concentration): 60 ppm N and 90 ppm P, Moderate nutrient (simulating pre-burning nutrient concentration): 43 ppm N and 63 ppm P, and Control: tap water. Different lower case letters denote significant differences among nutrient treatments within a node position or flower type and a species (P = 0.05).

The patterns of reproductive allocation to seeds as affected by nutrient treatments also contrasted between the 1st-2nd nodes and upper nodes seeds including CH seeds (Figs 5.8 A). Nutrients did not alter the reproductive allocation to CL seeds at the 1st-2nd nodes, but moderate nutrients increased the reproductive allocation to CH and CL seeds at the 6th node, while the high nutrient treatment increased reproductive allocation to CL seeds from the 3rd to the 6th nodes and CH seeds relative to the control (Fig. 5.8 A).

Reproductive allocation across nodes increased with nutrients only when CH seeds were included in the model ($P < 0.001$).

Nutrient conditions did not affect allocation to CL seeds in flechilla grande (Fig. 5.8, B), but allocation to CH seeds was greater ($P < 0.05$) in the control than in the moderate and high nutrient treatments. Therefore, this grass had a greater reproductive effort to produce CH seeds under low nutrients.

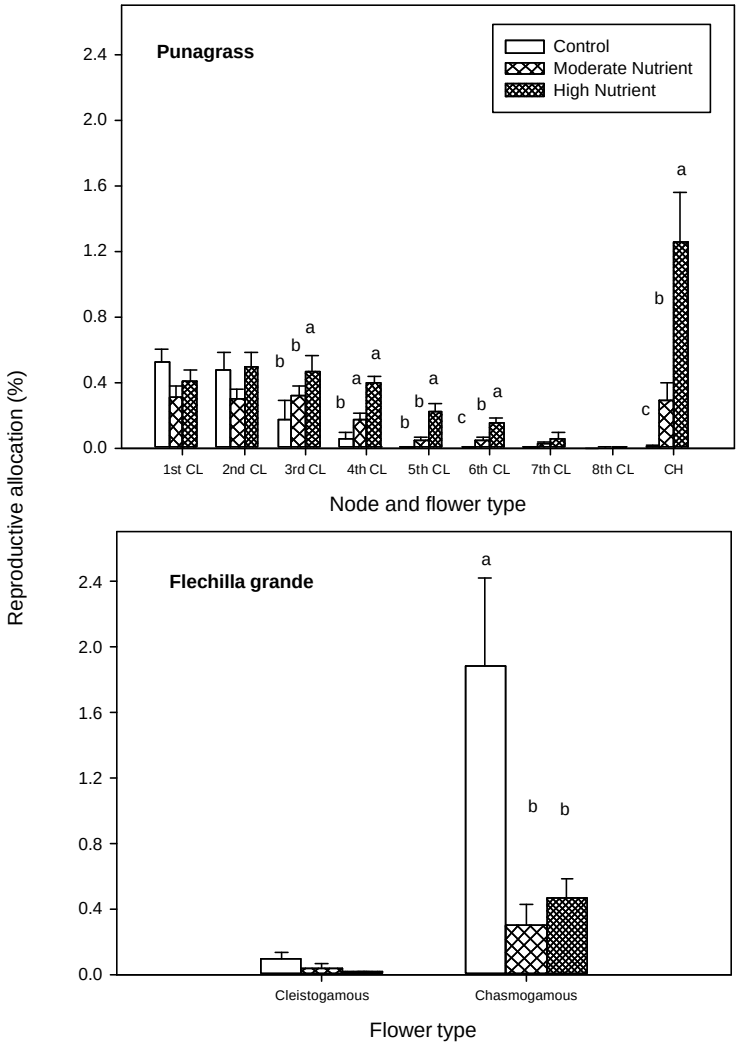

Figure 5.8 Effects of nutrient treatments on reproductive allocation to chasmogamous (CH) and cleistogamous (CL) seeds in each node in chasmousgamously derived plants of punagrass (*Achnatherum brachychaetum*) and flechilla grande (*Nassella clarazii*). Total biomass per plant includes total seed biomass, total tiller biomass and root biomass. High nutrient (simulating post-burning nutrient concentration): 60 ppm N and 90 ppm P, Moderate nutrient (simulating pre-burning nutrient concentration): 43 ppm N and 63 ppm P, and Control: tap water. Different lower case letters denote significant differences among nutrient treatments within a node position or flower type and a species ($P = 0.05$).

Production of CH and CL seeds per plant was positively correlated with plant size (Figs 5.9, 5.10) in punagrass. Larger plants produced more CH and CL seeds than smaller plants. There was a size weight threshold for plants to produce seeds, below which plants did not produce CH or CL seeds at the 4^{th}, 5^{th}, 6^{th} and 7^{th} nodes. The number of CL seeds per plant at the lowermost node (the 1^{st} node) (Fig 5.9) was less responsive to increasing levels of nutrients compared to seed number of the upper nodes including CH seeds.

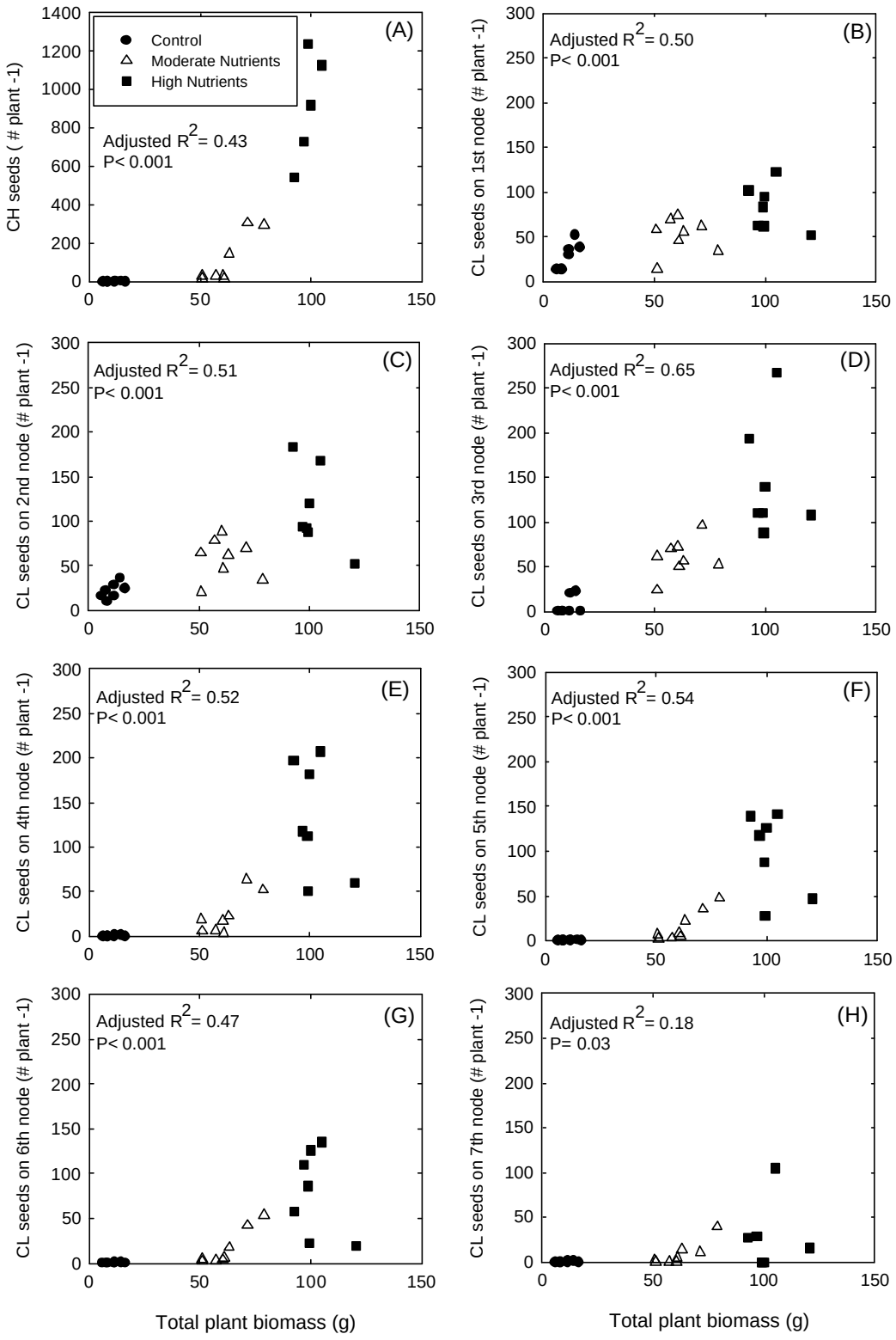

Figure 5.9 Relationship between seed number per node per plant and total plant biomass in punagrass (*Achnatherum brachychaetum*). (A) Chasmogamous (CH) seeds; (B) to (H) Cleistogamous (CL) seeds. High nutrient (simulating post-burning nutrient concentration): 60 ppm N and 90 ppm P, Moderate nutrient (simulating pre-burning nutrient concentration): 43 ppm N and 63 ppm P, and Control: tap water**.**

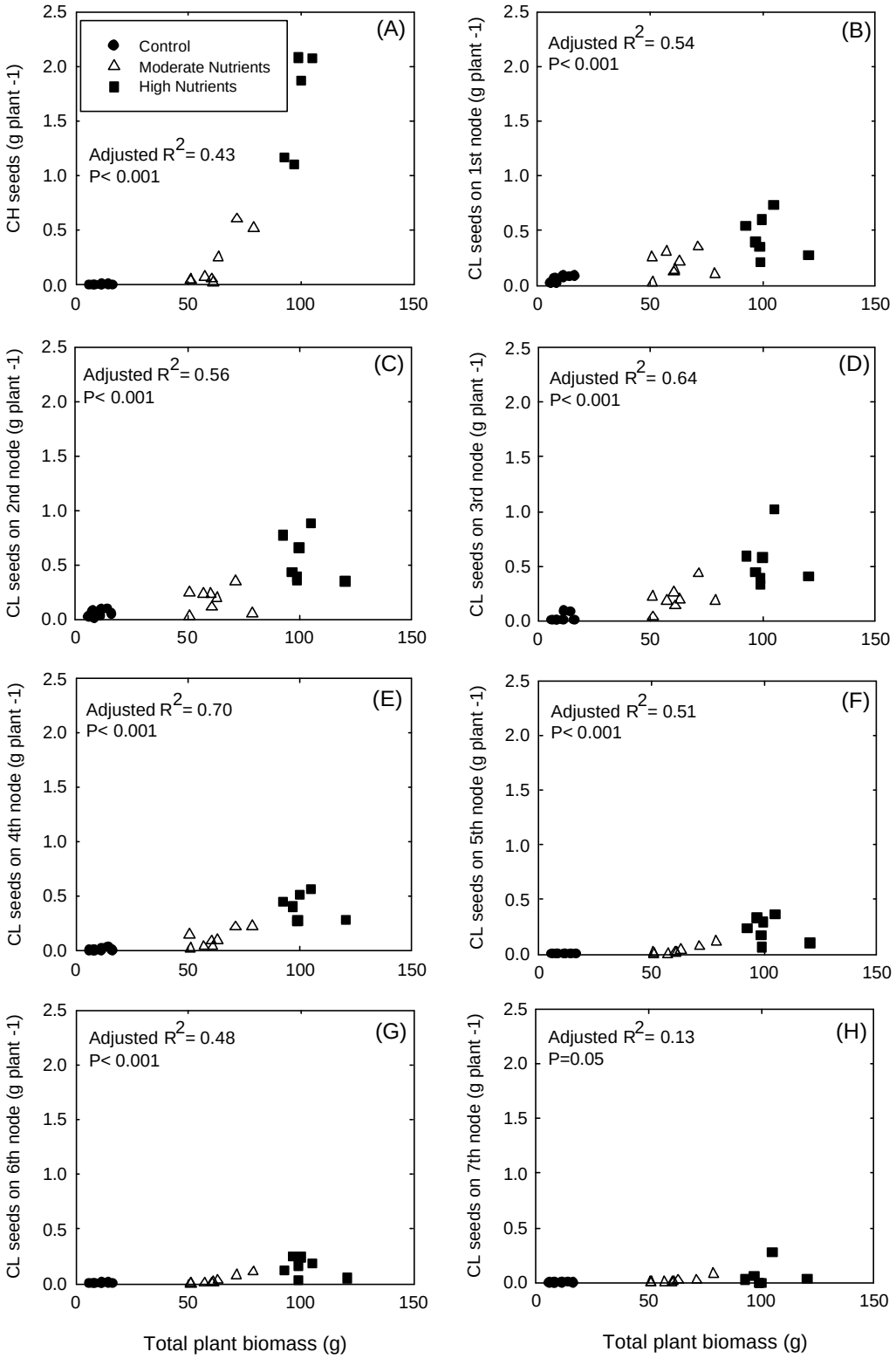

Figure 5.10 Relationship between seed mass per node per plant and total plant biomass in punagrass (*Achnatherum brachychaetum*). (A) Chasmogamous (CH) seeds; (B) to (H) Cleistogamous (CL) seeds. High nutrient (simulating post-burning nutrient concentration): 60 ppm N and 90 ppm P, Moderate nutrient (simulating pre-burning nutrient concentration): 43 ppm N and 63 ppm P, and Control: tap water.

For flechilla grande, chasmogamous and cleistogamous seed production was not correlated with plant size or maternal nutrient conditions (Fig. 5.11). Small plants of the control treatment produced CH and CL seeds.

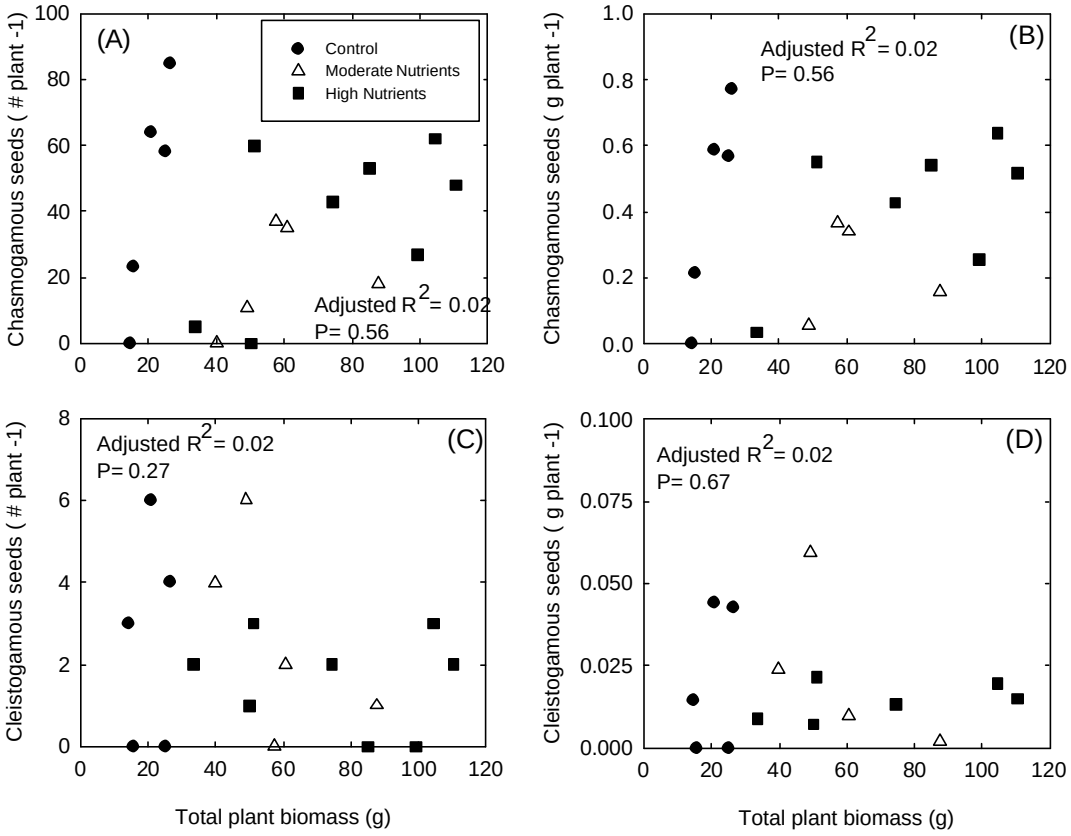

Figure 5.11 Relationship between chasmogamous (A-B) or cleistogamous (C-D) seed number or seed mass per plant and total plant biomass in flechilla grande (*Nassella clarazii*). High nutrient (simulating post-burning nutrient concentration): 60 ppm N and 90 ppm P, Moderate nutrient (simulating pre-burning nutrient concentration): 43 ppm N and 63 ppm P, and Control: tap water.

5.3.2 Effects of maternal nutrient environments on germination and viability of seeds maturing under greenhouse conditions in punagrass and flechilla grande

For punagrass, germination of seeds matured under greenhouse conditions was generally low (Fig. 5.12). Nevertheless, CH seeds produced under high nutrients had greater germination than those produced in the moderate nutrient treatment and the control. CL seeds produced at the 3rd node in the high nutrient treatment also had greater germination. Nutrient treatment did not affect germination of CL seeds on other nodes.

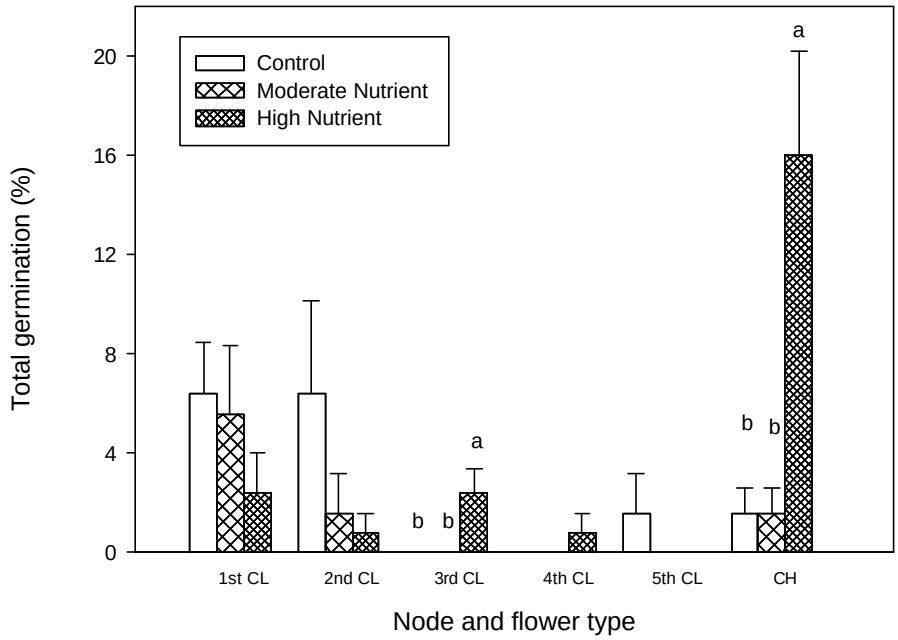

Figure 5.12 Maternal nutrient effects on the germination of chasmogamous (CH) and cleistogamous (CL) seeds maturated at different positions in punagrass (*Achnatherum brachychaetum*). High nutrient (simulating post-burning nutrient concentration): 60 ppm N and 90 ppm P, Moderate nutrient (simulating pre-burning nutrient concentration): 43 ppm N and 63 ppm P, and Control: tap water. For germination tests, seeds were incubated at 20/10° C, 9-h /15-h in darkness. Different lower case letters denote significant differences among nutrient treatments within a node position and flower type (P = 0.05).

A significant dehulling by nutrient effect ($P < 0.05$) was found for seed germination of flechilla grande (Fig. 5.13). Nutrients affected germination of seeds in the dehulling treatment ($P < 0.001$), but nutrients had no effect for the hulled seeds. Germination was greater in the high nutrient treatment than the control treatment.

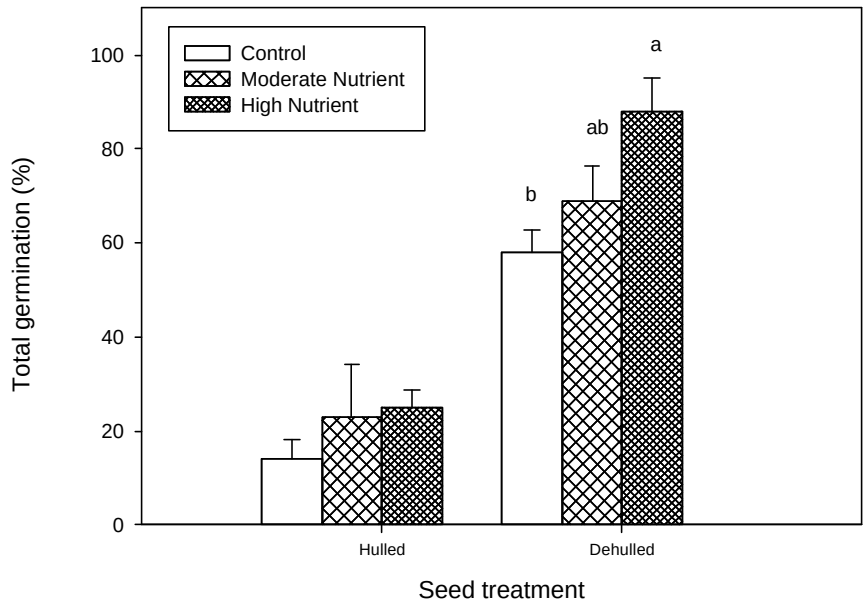

Figure 5.13 Maternal nutrient and dehulling effects on the germination of chasmogamous (CH) seeds in and flechilla grande (*Nassella clarazii*). High nutrient (simulating post-burning nutrient concentration): 60 ppm N and 90 ppm P, Moderate nutrient (simulating pre-burning nutrient concentration): 43 ppm N and 63 ppm P, and Control: tap water. For germination tests, seeds were incubated at 25/15° C, 9-h /15-h in light/darkness. Different lower case letters denote significant differences among nutrient treatments within a dehulling treatment (P = 0.05).

Seed viability tests revealed that most of the seeds were viable for both species (Fig. 5.14). The low nutrient treatment (control) reduced seed viability for the CL seeds of the 4th and 5th node of punagrass (Fig. 5.14 A). Viability of CH and CL seeds of flechilla grande was similar among nutrient treatments (Fig. 5.14 B).

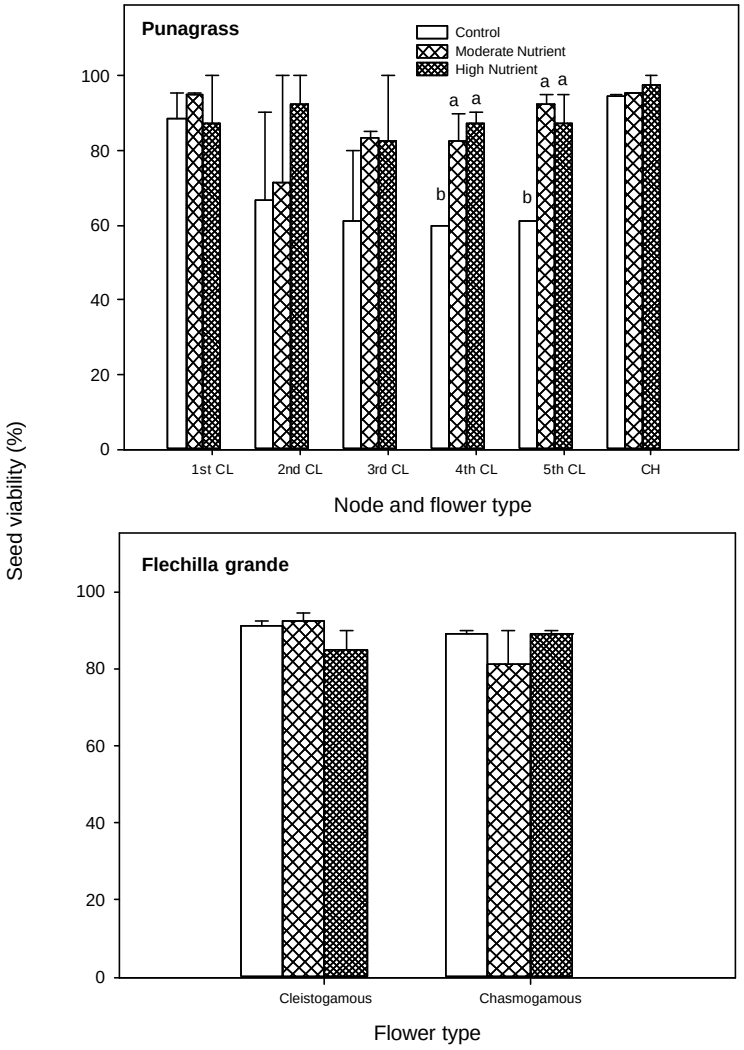

Figure 5.14 Maternal nutrient effects on the viability of chasmogamous (CH) and cleistogamous (CL) seeds maturated at different positions in punagrass (*Achnatherum brachychaetum*) and flechilla grande (*Nassella clarazii*). High nutrient (simulating post-burning nutrient concentration): 60 ppm N and 90 ppm P, Moderate nutrient (simulating pre-burning nutrient concentration): 43 ppm N and 63 ppm P, and Control: tap water. Different lower case letters denote significant differences among nutrient treatments within a node position or flower type and a species (P = 0.05).

5.4 Discussion

5.4.1 Effects of maternal nutrient environments on growth, seed production, biomass production and reproductive allocation to CH and CL seeds in punagrass and flechilla grande

The maternal nutrient environments in which plants were grown affected the vegetative and reproductive development, biomass and seed production in punagrass and flechilla grande. The two grasses responded differently to nutrients. Increasing nutrient availability enhanced leaf and tiller number, height, tiller and root biomass, and relative growth rate of height more in punagrass than in flechilla grande. This reflects differences in life history patterns between the two species. Punagrass and flechilla grande both grow in the caldén forest in central Argentina (Cano, 1988). Significant portions of the forest have been invaded by unpalatable, mid-grasses species, included punagrass. This invasion has been attributed to the interaction of overgrazing on palatable species by livestock, fire control and above-average rainfall during the last century (Distel and Bóo, 1995; Llorens, 1995; Busso, 1997; Llorens and Frank, 1999). Punagrass grows primarily in small patches beneath the caldén trees (Llorens, 1995) where nutrient availability can be high. Soil nutrients were greater under the canopy of caldén trees than in the open areas (Castelli and Lazzari, 2002). Flechilla grande, is a palatable grass associated with the dominant "climax" vegetation, grows on loamy soils in open areas. Flechilla grande is highly preferred by cattle and has a greater competitive ability when protected from grazing (Moretto and Distel, 1997) than unpalatable *Stipa* species. However, under conditions of selective and severe defoliation by livestock, unpalatable species such as punagrass gain competitive advantage (Moretto and Distel, 1999). In the present study, punagrass had several competitive advantages over flechilla grande, especially the high developmental rate, under nutrient conditions resembling high availability of resources after disturbances in grasslands. Under optimal environmental conditions, grasses from nutrient-poor habitats tend to have a lower growth potential than species from nutrient-rich habitats (Bradshaw *et al*, 1964; Lambers *et al*, 1998). The growth rate of slow –growing species from nutrient-poor habitats is less sensitive to the variation in the external supply of nutrients than one of fast-growing

species from nutrient-rich habitats (Garnier, 1998; Lambers *et al*, 1998). Late successional species have adaptations to mature stands where nutrients and other resources are limited and have less vegetative growth rates than early successional species (Tilman and Wedin, 1991).

The two grasses exhibited a trade-off between seed number and individual seed mass. Punagrass produced more, but smaller, seeds than flechilla grande. Seed size is positively correlated with seedling emergence, successful establishment and competitive ability (Rees, 1997; Leishman *et al*, 2000; Leishman, 2001). Increased competitiveness may be the primary selection pressure responsible for large seed mass (Leishman *et al*, 2000; Willson and Traveset, 2000). With increasing nutrient availability, reproductive allocation to CH seeds increased in punagrass but decreased in flechilla grande. Under limiting conditions, reproductive allocation was greater in flechilla grande than in punagrass. Reproductive allocation is greater in plants of open habitats than in species from of closed habitats (Bazzaz *et al*, 2000).

Plants are expected to alter seed number more than seed mass if resources vary (Smith and Fretwell, 1974). For species with seed heteromorphism, the relative quantities of the different seed types produced and their relative masses are influenced by environmental factors impacting the maternal plant (Weiss, 1980; Kawano *et al*, 1990; Cheplick, 1994; Kigel, 1995; Cheplick and Sung, 1998; Gutterman, 2000). Furthermore, resource constraints during seed provisioning can cause trade-off between seed number and mass, and contribute to seed mass variation within plants (Vaughton and Ramsey, 1998). Although seed number and individual seed mass were enhanced by increasing nutrient availability, high nutrients increased CH seed number and individual seed mass of CL seeds on the lowermost nodes. Increasing nutrients caused little change in seed number and individual seed mass in flechilla grande. Total seed mass per tiller was reduced by increasing nutrients for flechilla grande. Increases in CH seed number and inflorescence number with increasing soil fertility have been reported in *A. purshii* Kunts (Cheplick, 1989), and *T. purpurea* (Walt.) Chapm. (Cheplick, 1996b; Cheplick and Sung, 1998), and *Calathea micans* L. Mathieu (Le Corff, 1993). CL seed mass also increased with soil fertility in *T. purpurea* (Cheplick, 1996b; Cheplick and Sung, 1998).

Altering the relative size and number of offspring in response to the prevailing conditions is critical to fitness (Venable, 1992; Kigel, 1995).

Reproductive allocation to CL seeds on the two lowermost nodes in punagrass was constant among nutrient treatments, which may help ensure the production of a consistent number of large seeds at the basal nodes in an uncertain environment (Schoen and Lloyd, 1984). Under low nutrient conditions, punagrass allocated more resources to CL seeds on the two lowermost nodes than to CH seeds. More resources were allocated to CL than to CH seeds in grasses under limited resources (McNamara and Quinn, 1977; Cheplick and Quinn, 1982). Allocation to CL, subterranean seeds was fairly constant at the two nutrient levels studied, while allocation to aerial CH seeds increased with high nutrients in *Ampicarpum purshii* (Cheplick 1989). Under relatively poor environmental conditions, the greatest fitness results from economically produced CL seeds; however, if environmental conditions are favourable, the greatest fitness is reflected in increased production of the more costly CH seeds that can be dispersed away and occupy areas with less competition (Schoen and Lloyd, 1984; Quinn, 1998).

Seed production in punagrass in response to increasing nutrients was positively correlated with vegetative growth. Plants of punagrass reached a certain size before producing seeds on the upper nodes including CH seeds of the terminal panicles. The extent of the reproductive mode is dependent on nutrient conditions through their effects on plant growth and size. Cleistogamy of punagrass at the lowest nodes under low nutrients allows small plants to reproduce in unfavourable conditions. Increased CH flower production is a function of plant size as affected by nutrients (Cheplick, 1989). Plant size effect had no effect on the type of reproduction in *Calathea micans* L. Mathieu, but increased nutrients led to more shoots being produced with a higher probability of producing CH inflorescences (Le Corff, 1993).

5.4.2 Effects of maternal nutrient environments on germination and viability of seeds maturing under greenhouse conditions in punagrass and flechilla grande

Maternal nutrient environments increased germinability of punagrass and flechilla grande seeds. High nutrient for maternal plants enhance seed germinability in many species (Fenner, 1991), such as tomato (George *et al*, 1980; Varis and George,

1985), tobacco (*Nicotiana tabacum* L. Tobacco*)* (Thomas and Raper, 1979), tall fescue (*Festuca arundinacea* Schreb.) (Watson and Watson, 1982), lambsquarters (*Chenopodium album* L.) (Fawcet and Slife, 1978) and purple sandgrass (*T. purpurea*) (Cheplick and Sung, 1998). Germination more than doubled when high amounts of nitrogen and phosphorus were applied to maternal plants of tomato. Seed dorma ncy was greater in punagrass than in flechilla grande seeds. Maternal nutrients had little effect on the germination of CL seeds in punagrass. High nutrients, but not moderate nutrients, increased germination of CH seeds from about 2% to 16% in punagrass. Maternal nutrients did not alter the germination of intact CH seeds with hulls in flechilla grande, but germination was increased from 58% to 88% under high nutrients when the palea and lemma were removed. Greater germination with increasing nutrients was observed in *T. purpurea* (Cheplick and Sung, 1998), and was explained by the increased seed mass and nutrient content (Parrish and Bazzaz, 1985; Cheplick and Sung, 1998; Vaughton and Ramsey, 1998). Increasing seed mass in punagrass with increased maternal nutrients does not necessarily increase germination of CL seeds.

The position of seeds on a plant can affect their germination (Datta *et al*, 1972; Simpson, 1990, Gonzalez Rabanal *et al*, 1994; Kigel, 1995; Cheplick, 1996a; Gutterman, 2000). Seeds produced by cleistogamous and chasmogamous flowers of grasses may vary in germinability (McNamara and Quinn, 1977; Bell and Quinn, 1985, Baskin and Baskin, 1998, Cheplick and Sung, 1998). The nutrient environment for maternal plants carries over, influencing germination in punagrass and flechilla grande. Moreover, the nutrient environment of maternal plants affected germination in a seed type-dependent pattern for punagrass, with more impact on CH than CL seeds.

5.4.3 Summary

Favourable nutrient environments enhance growth, development, biomass and seed production more in punagrass than in flechilla grande. Punagrass behaves as a fast-growing species from nutrient-rich habitats and flechilla grande behaves like a slow-growing species from nutrient-poor habitats.

Flechilla grande grows in open areas of the grasslands where nutrients are more limiting. Under these conditions, this grass is able to allocate resources to produce a few large CH seeds with high potential for seedling competition. Although increasing

nutrient increased the mass of seeds on the lowermost nodes in punagrass, reproductive allocation to these seeds was constant among nutrient treatments. Furthermore, under limited nutrients punagrass allocates more resources to produce CL seeds on the lowermost nodes while producing few CH seeds. When resources are abundant, punagrass reallocates resources to produce many small CH seeds with high dispersal potential. Increasing nutrient enhanced seed production and germinability of CH seeds in punagrass and germinability of CH seeds of flechilla grande after hulls were removed. The production of many small CH seeds with higher germinability in punagrass has ecological consequences in terms of increased dispersal potential. Production of a few large CH seeds in flechilla grande confers competitive advantages. The response of punagrass and flechilla grande increases our understanding of the effect of maternal environmental conditions on the reproductive strategies of unpalatable and palatable species of grasslands in Argentina.

6. GENERAL DISCUSSION AND CONCLUSIONS

The present studies suggest that chasmogamous and cleistogamous seeds have different roles in the fitness of punagrass and flechilla grande. The null hypothesis that seed heteromorphism plays similar roles in the fitness of two species with different functional and ecological characteristics is rejected. The findings of this thesis reveal the importance of studying adaptive variation in reproductive strategies, reflecting both habitats and life-history patterns. Generally, grasses display an extraordinary diversity of reproductive strategies in response to environmental variation (Quinn, 2000).

6.1 Contrasting heteromorphism of chasmogamous and cleistogamous seeds in punagrass and flechilla grande

In both species, the ecological significance of having seed heteromorphism is that sibling competition is probably reduced by having more diverse offspring. Variable progeny is predicted to have a greater ability for partition of limiting resources (Cheplick, 2004).

CL seeds of punagrass are produced on all nodes of tillers. Seed mass decreased from the bottom to the top of tillers with CH seeds being the smallest. Seed size of CL seed size increased in areas with competition from palatable grasses and by increasing maternal nutrient environments, whereas seed size of CH seeds remained unaffected. Moreover, the effect of range condition on CL seed size was also manifested when progeny plants were grown under controlled conditions in the greenhouse. Seed dispersal is more effective when seed mass is low (Westoby *et al*, 1996; Rees, 1997), but seedlings that are more competitive with a better chance of survival develop from the larger CL seeds (McNamara and Quinn, 1977; Cheplick and Quinn, 1982; Cheplick, 1994; Cheplick and Wickstrom, 1999). Punagrass grows in small patches beneath trees in grasslands with high proportion of palatable grasses (Llorens, 1995) where nutrient availability is greater than in open areas (Castelli and Lazzari, 2002). It is probable that

punagrass increases seed mass of CL seeds in patches of high soil fertility, provisioning better to seeds which are least likely to disperse in space and most likely to experience sibling competition. The size of CH seeds do not change with nutrients allowing effective seed dispersal.

Chasmogamous seeds had a greater germination than cleistogamous seeds of punagrass. Germination was greater in CH seeds from grasslands in good condition than in overgrazed grasslands, and in seeds produced in greenhouse when plants were grown in high nutrient conditions. Patches with high soil fertility seem to provide favourable, nutrient environments for punagrass, increasing the fitness of the maternal plants through improved germinability of CH seeds with high dispersal potential. On the other hand, poor range condition and increasing nutrient environments for maternal plants did not change the dormancy of the CL seeds, indicating that CL seeds of punagrass can form a persistent seed bank under various environmental conditions. Dormancy in CL seeds ensures seedling emergence over time, thus reducing local crowding (Nilsson *et al*, 1994; Kobayashi and Yamamura; Olivieri, 2002). On the other hand, outcross breeding systems may have been selected to minimize sibling competition. Thus, dispersal of CH seeds and dormancy of CL seeds in punagrass have similar ecological significance in reducing sibling competition in patches with high soil fertility.

Chasmogamous and cleistogamous seeds of flechilla grande did not consistently differ in size and dormancy, and removal of hulls enhanced germination of both seed types similarly. Maternal nutrient environments had minimal effects on seed size and germination of CH seeds in flechilla grande. Flechilla grande is associated with the climax vegetation, occupying open areas of the caldén forest of central Argentina where nutrients are limiting (Llorens, 1995; Castelli and Lazzari, 2002). This species is more competitive than unpalatable grasses when protected from grazing. For example, flechilla grande has greater nutrient uptake than unpalatable species (Saint-Pierre *et al*, 2004). Both seed types in flechilla grande contributed similarly to adult plant traits. Competition for resources among CH progeny was not as great as among CL progeny in *D. clandestinum*, a species with CH and CL seeds of similar size (Bell and Quinn, 1985). An advantage of having CH and CL seeds in flechilla grande may be to have more variable progeny that in turn reduces potential competition among siblings.

Seeds of flechilla grande with mechanisms for delaying germination (physical dormancy), dispersal and/or burial (hygroscopic awns), as well a limited seed production can be selected for partition of limited resources, while minimizing intraspecific and interspecific competition. Moreover, the production of CL seeds in punagrass and flechilla grande permits a faithful transmission of successful parental genomes to progeny through selfing, which in turn may confer high fitness in specific environmental conditions (Schoen and Lloyd 1984).

Seed size of CH seeds was greater in flechilla grande than in punagrass, but the size of CL seeds was comparable between the two species, reflecting differences in colonization/competition strategies between the two species and between CH and CL seeds. Small CH seeds of punagrass confer adaptation for colonizing disturbed habitats, while large CH seeds of flechilla grande and large CL seeds of both species can increase the competitive ability. Increased competitiveness may be the primary selection pressure responsible for large seed mass (Leishman *et al*, 2000; Willson and Traveset, 2000). Nevertheless, plants of punagrass can produce seeds that are as large as those of flechilla grande only under favorable nutrient conditions for maternal plants. Overall, flechilla grande has larger seeds and high germination, reflecting adaptations to unproductive habitats while punagrass shows regenerative characteristics that are adaptive for sites with fertile soils.

6.2 Differential fitness of chasmogamous and cleistogamous seeds in punagrass and flechilla grande

CH progeny plants of punagrass grew and developed fast, but they had low competitive ability. The growth rate, development, biomass and seed production of punagrass were enhanced by increasing nutrient, displaying adaptations to nutrient-rich environments. CL progeny of punagrass grew slower than CH progeny at the vegetative stage, but then the CL progeny allocated more resources and produced more CH seeds. CL progeny of punagrass may persist in mature plant communities as a source of CH propagules until the creation of gaps, which favours growth and development of the less competitive CH progeny. Seedling emergence and establishment of unpalatable *Stipa spp*. were greatest in microsites without competition from palatable species (Moretto and Distel, 1998).

Persistence of punagrass in the soil seed bank can also explain the stability of punagrass patches. When unpalatable grasses gain dominance, it is difficult to reverse this process even if grazing is reduced or removed (Llorens, 1995). On the other hand, CH progeny of punagrass produced more and heavier CL seeds and allocated more resources to produce CL seeds in plants from grassland in good condition than from poor range condition. CH progeny allocated resources to CL seeds of the lowermost nodes, but produced few CH seeds when maternal plants were grown in low nutrients. Thus, severe competition can be a short-term selective force on plant fitness of punagrass, by increasing the production of seeds with high potential for persistence in late seral plant communities. However, when resources are abundant, punagrass reallocates resources to produce more small, CH seeds. Both progeny of punagrass are probably able to respond to increasing nutrients by producing more CH seeds, but this capacity can be superior for CL progeny, which produced more CH seeds and allocated more resources in CH seeds than CH progeny. Therefore, chasmogamous and cleistogamous seeds of punagrass have different contributions to fitness under different environmental conditions.

Flechilla grande produced few large CH seeds with devices for burial. Flechilla grande also produced few CL seeds that can remain close to the maternal plant. In many plants adapted to semi-arid and arid conditions, few seeds that are dispersed close to the maternal plant increases chances for germination and reproduction, while minimising sibling competition (Kigel, 1995). Under limiting resources, punagrass and flechilla grande allocated resources to increase seedling competition. For example, flechilla grande produced a few large CH seeds whereas punagrass produced large CL seeds at the lowermost nodes but only a few CH seeds. Limited production of large CH and CL seeds in flechilla grande as compared to punagrass may confer superior competitive ability in local conditions, at the same time this strategy may be disadvantageous under heavy grazing, which favours species such as punagrass with many small CH seeds, high potential for colonization as well as large seeds for seedling competition.

Punagras and flechilla grande also differ in their patterns of cleistogamy. Punagrass produces CL seeds on all the nodes of the stem, whereas flechilla grande develops CL seeds at the base of plant. The production of CL seeds in both species may

be adaptive for grazing or fires (Campbell *et al*, 1983). Grazers are more likely to eat CH seeds from the upper portion of *Stipa leucotricha* than CL seeds located in the leaf sheath at the base of the plant (Dyksterhuis, 1945). However, selective grazing by cattle on flechilla grande reduces its dominance and favours unpalatable species such as punagrass. Nevertheless, production of CL seeds is crucial for the regeneration of flechilla grande in grasslands of central Argentina.

The information derived from this research is important to rangeland managers and government agencies for restoring degraded grasslands in central Argentina. Long periods of resting from grazing to improve range condition of degraded areas may not be the best management approach if the soil seed bank is composed of many CL seeds and the herbaceous layer has a high density of adult plants of punagrass. Range management measurements should be directed toward reducing soil seed inputs and reducing vigour of punagrass at early stages of development to affect seed production. Since cattle can graze mid-grasses following fire, prescribed burning at the end of the summer followed by high stocking rates in grasslands dominated by unpalatable species can restore conditions for development of palatable species. Nevertheless, it is difficult that better grazing management, stocking rate adjustments and fire management could enhance the natural regeneration of flechilla grande because of its low seed production. In this sense, reseeding with flechilla grande is recommended. Further research on safe sites for CH and CL seeds, the effect of magnitude, timing and frequency of disturbances on gaps dynamics, and patterns of seed dispersal is needed to allow a reseeding program for flechilla grande.

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APPENDIX

Table A1 Effects of progeny on vegetative attributes of punagrass (*Achnatherum brachychaetum*). P_{CL}: plants derived from cleistogamously produced seeds on the 1st , 3rd and 5th node of the tiller. P_{CH}: plants derived from Top chasmousgamously produced seeds. DAP: days after planting.

Attribute	P_{CL} 1st	P_{CL} 3rd	P_{CL} 5th	P_{CH} Top
Tillers with CH seeds per plant at 91 DAP	123	142	124	89
Height (cm) at 91 DAP	161	100	128	100
Leaves per plant at 91 DAP	304	375	361	242
Average tiller biomass (g)	1.63	1.1	1.54	1.64
Average root biomass (g)	11.9	11.3	13.9	25.0

Table A2 Effects of progeny on vegetative attributes of flechilla grande *(Nassella clarazii*). P_{CL}: plants derived from cleistogamously produced seeds. P_{CH}: plants derived from chasmousgamously produced seeds. DAP: days after planting.

Attribute	P_{CL}	P_{CH}
Relative Growth Rate of height at 76 DAP ($cm\,cm^{-1}\,d^{-1}$)	0.023	0.029
Tillers per plant at 91 DAP	70	54
Leaves per plant at 91 DAP	185	153
Average tiller biomass (g)	1.9	2.2
Average root biomass (g)	5.88	5.35

Table A3 Effects of site ecological history on vegetative attributes of Chasmousgamously derived plants of punagrass (*Achnatherum brachychaetum*) from seeds collected in areas of contrasting range conditions (Poor and Good). DAP: days after planting.

Attribute	Range condition	
	Poor	**Good**
Relative Growth Rate of height at 29 DAP(cm cm^{-1} d^{-1})	0.062	0.066
Tillers per plant	38	45
Leaves per plant	105	128
Average tiller biomass (g)	1.3	1.3
Average root biomass (g)	9.5	9.3

Table A4 Effects of progeny on reproductive attributes of punagrass (*Achnatherum brachychaetum*). P_{CL}: plants derived from cleistogamously produced seeds on the 1st, 5th and 7th node of the tiller. P_{CH}: plants derived from Top chasmousgamously produced seeds,

Attribute	P_{CL} 1st	P_{CL} 3rd	P_{CL} 5th	P_{CH} Top
Tillers with CH seeds per plant at harvest	31.5	29.8	33	28.4
Seeds per tiller	322	185	234	256
CH seeds per tiller	256	138	181	198
CL seeds per tiller	66	48	53	58
Seed mass per tiller (mg)	665	333	457	586
CH seed mass per tiller (mg)	463	215	286	385
CL seed mass per tiller (mg)	201	118	171	201
Seeds per plant	8,610	5,726	5,390	7,192
CH seeds per plant	6,309	3,507	3,510	4,933
CL seeds per plant	2,301	2,220	1,880	2,259
Seed mass per plant (mg)	18,983	11,225	12,856	18,062
CH seed mass per plant (mg)	11,401	5,434	5,523	9,667
CL seed mass per plant (mg)	7,582	5,791	7,334	8,395

Table A5 Effects of progeny on reproductive attributes of plants of flechilla grande *(Nassella clarazii*). P_{CL}: plants derived from cleistogamously produced seeds. P_{CH}: plants derived from chasmousgamously produced seeds. DAP: days after planting.

Attribute	P_{CL}	P_{CH}
Tiller with CH seeds per plant at harvest	8	11
Tillers with CH seeds per plant at harvest (%)	10	17
Seeds per tiller	6	6
CH seeds per tiller	6	6
CL seeds per tiller	0	0
Seed mass per tiller (mg)	50.8	85.0
CH seed mass per tiller	50.5	80.5
CL seed mass per tiller	0.3	4.5
Seeds per plant	42	45
CH seeds per plant	42	42
CL seeds per plant	1	2
Seed mass per plant (mg)	253.8	425
CH seed mass per plant (mg)	252.4	402.6
CL seed mass per plant (mg)	1.4	22.4
Reproductive allocation to CH seeds (%)	0.15	0.28
Reproductive allocation to CL seeds (%)	0.00	0.02
Germination of CH seeds of offspring (%)	19	10

Table A6 Effects of site ecological history on reproductive attributes of Chasmousgamously derived plants of punagrass (*Achnatherum brachychaetum*) from seeds collected in areas of contrasting range conditions (Poor and Good). CH: chasmogamous, CL: cleistogamous.

Attribute	Range condition	
	Poor	**Good**
Tillers per plant with CH seeds at harvest (%)	39	41
Seeds per tiller	204	197
CH seed per tiller	154	142
CL seeds per tiller	50	55
Seed mass per tiller (mg)	558	497
CH seed mass per tiller	373	292
CL seed mass per tiller	185	205
Seeds per plant	3417	4824
CH seeds per plant	1976	2761
Seed mass per plant (mg)	10450	13970
CH seed mass per plant (mg)	4708	5656